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A Method for the Rapid Determination of Salicylates.

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In the course of some work on the gastric and intestinal absorption of salicylates, existing methods of determining salicylates were found inadequate. Bromine titration methods¹ used on pure solutions of salicylates are not applicable to biological solutions which contain benzene rings. Like Hanzlik,² we found extraction with immiscible solvents to be incomplete. The method of Hanzlik and Presho³ was found unsatisfactory for even after five or six hours of steam distillation from phosphoric acid, salicylic acid was found in the distillate. Furthermore, the depth of color developed with ferric chloride varied with the pH and the time elapsing before the colorimetric reading was made.

The method finally devised depends upon measuring the volume of carbon dioxide liberated by brominating salicylic acid in the Van

* P represents a preliminary, C a complete manuscript.

¹ Seidell, *J. Am. Chem. Soc.*, 1909, **31**, 1169.

² Thoburn and Hanzlik, *J. Biol. Chem.*, 1915, **23**, 163.

³ Hanzlik and Presho, *J. Pharm. Exp. Therap.*, 1923, **21**, 247.

Slyke volumetric gas analysis apparatus.⁴ This method was successfully used in this laboratory in determining gastric and intestinal absorption of acetylsalicylic acid, salicylic acid and its salts and mixtures of these compounds with calcium gluconate, sodium bicarbonate, and magnesium oxide.⁵

Solutions. Saturated Na Br solution saturated with Br₂; saturated KI; 1:4 sulfuric acid.

A known volume (10 cc. or less) of the solution containing between 1 and 5 mg. of salicylate as salicylic acid is used. In the case of acetylsalicylic acid or other combined salicylates and urine, in which a large percentage of salicylate is excreted as salicyluric acid, hydrolysis is necessary. Hydrolysis is accomplished by heating in the steam bath with 1 drop of concentrated NaOH for 1 hour. The solution is then acidified with 1:4 sulfuric acid and washed into the chamber of the Van Slyke apparatus. Preformed CO₂ and dissolved air are removed by evacuating the apparatus in the usual way, shaking and then forcing the gas out through the cup. After complete elimination of preformed gas, the levelling bulb is raised so that the upper surface of the solution is at the top of the upper stop cock. The bulb is placed on the lower ring and $\frac{1}{4}$ - $\frac{1}{2}$ cc. of the Br₂ solution is run in from the cup by opening the upper stop cock. The two solutions are mixed and allowed to stand 1 minute. Then $\frac{1}{4}$ - $\frac{1}{2}$ cc. KI solution is run in so that the excess Br₂ will replace the I⁻ of the KI with liberation of I₂. This is done so that correction for the vapor pressure of Br₂ will not have to be made. The vapor pressure of I₂ is so small at room temperature that it does not have to be taken into consideration.

Salicylic acid is readily brominated with the formation of tribromophenol and CO₂. One gram mole of salicylic acid liberates one gram mole or 22.4 liters of CO₂. Therefore, 1 cc. of CO₂ at standard conditions is equivalent to 6.15 mg. salicylic acid and the number of mg. of salicylate as salicylic acid in the sample used is obtained by multiplying the reading of the apparatus in cc. reduced to standard conditions by 6.15 or by 8.03 for values in terms of acetylsalicylic acid.

Results. This method was found to give satisfactory results on samples of urine and gastric juice to which known quantities of salicylate were added as well as pure solutions of salicylates in water. Table I shows values obtained upon known solutions of acetylsali-

⁴ Van Slyke and Cullen, *J. Biol. Chem.*, 1917, **30**, 289; Van Slyke, *J. Biol. Chem.*, 1917, **30**, 347; Van Slyke and Stadie, *J. Biol. Chem.*, 1921, **49**, 1.

⁵ Bradley, Schnedorf and Ivy, *J. Dig. Dis. and Nut.*, 1936, **3**, 415.

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TABLE I.
Normal Urine Plus Acetylsalicylic Acid or Na Salicylate.

| Cc. sample | Reading of Van Slyke | Barometric Pres. | Temp., C° | Correction factor | Amt. found, mg. | Amt. actually added, mg. | % error |
|------------|-------------------------|---------------------|-----------|----------------------|--------------------|-----------------------------|---------|
| 10 | .67 | 746 | 26 | .862 | 4.12 | 4.00 | 3.0 |
| 10 | .67 | 746 | 26 | .862 | 4.12 | 4.00 | 3.0 |
| 5 | .34 | 746 | 26 | .862 | 2.09 | 2.00 | 4.2 |
| 5 | .34 | 746 | 26 | .862 | 2.09 | 2.00 | 4.2 |
| 5 | .751 | 744-500 | 28 | .259 | 1.56 | 1.50 | 4.0 |
| 5 | .748 | 744-500 | 28 | .259 | 1.55 | 1.50 | 3.7 |
| 5 | .751 | 744-500 | 28 | .259 | 1.56 | 1.50 | 4.0 |
| 10 | .44 | 744 | 28 | .850 | 3.00 | 3.00 | 0.0 |
| 10 | .44 | 744 | 28 | .850 | 3.00 | 3.00 | 0.0 |
| 5 | .420 | 743 | 27 | .855 | 2.56 | 2.5 | 2.4 |
| 5 | .420 | 743 | 27 | .855 | 2.56 | 2.5 | 2.4 |
| 5 | .480 | 743 | 27 | .855 | 2.93 | 3.0 | 2.3 |
| 5 | .485 | 743 | 27 | .855 | 2.96 | 3.0 | 1.3 |

cyclic acid and sodium salicylate in urine. As can be seen, the amount found by analysis checks very well with the quantity added, the percentage error ranging from 0-4%.

Urine from a subject who was given 15 grains of acetylsalicylic acid was analyzed to determine the amount of salicylate present. Then known quantities of acetylsalicylic acid were added and the

TABLE II.
Urine from Subject After Taking 15 gm. Aspirin.

| Cc. sample | Reading Van Slyke | Amount Found | Amount per cc. | Barometric Pressure 745 Temp. 30° |
|------------|----------------------|-----------------|-------------------|--------------------------------------|
| 10 | .400 | 2.73 | .273 | |
| 10 | .401 | 2.74 | .274 | Aver. .274 mg./cc. |
| 10 | .403 | 2.75 | .275 | |

Same Urine to Which Acetylsalicylic Acid Was Added.

| Cc. sample | Reading Van Slyke | mg./cc. Pres. Orig. | mg./cc. Found | mg./cc. Added | mg./cc. Recovered | % error |
|------------|----------------------|------------------------|------------------|------------------|----------------------|---------|
| 5 | 491 | .274 | .671 | .400 | .397 | .8 |
| 5 | 492 | .274 | .672 | .400 | .398 | .5 |
| 5 | 491 | .274 | .671 | .400 | .397 | .8 |
| 5 | 440 | .274 | .601 | .320 | .327 | .2 |
| 5 | 440 | .274 | .601 | .320 | .327 | .2 |
| 10 | .680 | .274 | .473 | .200 | .199 | .5 |
| 10 | .681 | .278 | .474 | .200 | .200 | .0 |

analyses were repeated. As seen in Table II, the amounts determined check very well with the amount added.

Known quantities of acetylsalicylic acid were added to gastric juice collected from a Pavlov pouch dog and samples analyzed. Here

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again analyses checked with actual quantities added to within 0.5-4.0%.

An attempt was made to apply this method to blood without success, for, if hydrolysis is attempted on the blood itself, a mass of denatured protein results, and attempts using blood filtrates were disappointing because of the absorption of the salicylate by the protein precipitate.

TABLE III.
Solutions of Acetylsalicylic Acid in Gastric Juice.

| Ce. sample | Reading Van Slyke | Mg. found | Mg. added | % error |
|------------|----------------------|-----------|-----------|---------|
| 5 | .410 | 2.90 | 3 | 3 |
| 5 | .405 | 2.86 | 3 | 4 |
| 5 | .415 | 2.93 | 3 | 2 |
| 5 | .280 | 1.98 | 2 | 1 |
| 5 | .280 | 1.98 | 2 | 1 |
| 5 | .285 | 2.01 | 2 | 0.5 |

The sources of error entering into this method are those that enter into any method depending upon the measurement of gas volumes over aqueous solutions. The apparatus must, of course, be tight at all its stop cocks. The liberation of gas must be complete and, in order to accomplish this, the chamber must be shaken vigorously. The high concentration of salts facilitates the liberation of CO₂ and also hinders the absorption of the gas when the mercury is levelled, thus reducing the inaccuracy at this stage of the procedure.

Conclusion. This method enables one to analyze rapidly for salicylates in biological fluids in which there are no suspended solids to occlude the apparatus. The time required is short—one hour for the hydrolysis, the time necessary for the actual analysis depending upon the skill developed by the operator of the Van Slyke apparatus. The accuracy of this method was found to exceed that of other existing methods and does not depend upon one's ability to match colors in a colorimeter.

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Observations on the Kurloff Bodies in the Blood of Guinea Pigs.

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Kurloff¹ first described granular inclusions within the cytoplasm of what he called lymphocytes in guinea pigs' blood. It was shown subsequently that the cells containing these bodies were monocytes.² Wright's stained blood smears reveal the Kurloff bodies as dark red, homogeneous masses in the cytoplasm. Although many workers have observed and studied these inclusions, little is known concerning their origin and function. They appear to be found only in the blood of guinea pigs.

Bender and DeWitt,³ while studying the blood picture of tuberculous guinea pigs, observed that their normal control pigs showed an unexpected eosinophilia. At the same time, Kurloff bodies appeared in the blood. They decided that there was a relationship between eosinophilia and Kurloff bodies. They were able to reduce the eosinophiles with mercurial medication, but not the number of Kurloff bodies.

During an investigation⁴ of eosinophilia in trichinous guinea pigs about 3 months old obtained from one source, I observed the number of Kurloff bodies in several hundred Wright's stained blood smears. The cells were counted 3 times a week for 6 to 8 weeks. All the animals had Kurloff bodies in their blood when first examined. Six normal control pigs had varying levels of eosinophiles. There was a definite, direct relationship both in the healthy and infected pigs between the number of eosinophile cells and the number of Kurloff bodies in the monocytes so that the high levels of eosinophile cells were accompanied by the greatest number of Kurloff bodies. It was possible to reduce markedly the number of eosinophile cells in trichinous animals by a staphylococcus infection, but this did not essentially affect the number of Kurloff bodies. Eight trichinous animals injected with virulent tubercle bacilli showed the same tendency for a reduction in eosinophiles, while the Kurloff bodies maintained their original level.

¹ Kurloff, cited by P. Ehrlich and A. Lazarus in *Specielle Pathologie u. Therapie*, edited by H. Nothnagel, Wien, 1898, VIII, 56.

² Bender, L., *J. Med. Research*, 1934, 44, 383.

³ Bender, L., and DeWitt, L. M., *Am. Rev. Tuber.*, 1923-24, 8, 138.

⁴ Spink, W. W., *Arch. Int. Med.*, 1934, 54, 805.

Of considerable interest were the findings in animals infected with the protozoa, *Trypanosoma equiperdum*. Although these animals maintained a moderately high level of eosinophile cells, there were few or no Kurloff bodies in their blood. This may be explained by the fact that in this infection there is a lymphocytosis and the monocytes are few. This relationship between the level of the monocytes and Kurloff bodies was not constant in all of the preceding animals mentioned.

These observations not only confirm the work of Benedict and De Witt, but also demonstrate that an acute bacterial infection in guinea pigs can reduce eosinophile cells but not the number of Kurloff bodies. However, if an infection results in reducing the number of monocytes as in Trypanosomiasis, the Kurloff bodies are also reduced and may become absent. In conclusion, although there appears to be a numerical relationship between the eosinophile cells and Kurloff bodies in guinea pigs' blood, the absolute number of Kurloff bodies is dependent upon the level of the monocytes.

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Reciprocal Relationship of Copper and Iron in Blood. Polycythemia Vera.

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We have already reported the blood iron content of the adult to be an average of 50 mg. per 100 cc. for men and 45 mg. per 100 cc. for women, and the blood copper 0.132 mg. or 132 micrograms per 100 cc. for men and women.^{1, 2} In determining the blood iron and copper in various pathological conditions accompanied by anemia, we observed an inverse relationship between the copper and the iron in the blood. As the blood iron fell, the blood copper rose. Hypercupremia was the usual response to hypoferronemia.^{1, 2}

We have already reported the blood findings in a white woman, age 60, suffering from polycythemia vera.² We now report another case which verifies our first findings. The aim of the treatment in vogue in this condition, which is manifested by an increase in the red cell count beyond the normal, is to cut down the number of red cells

¹ Sachs, A., Levine, V. E., and Appelsis, A., *Arch. Int. Med.*, 1933, **52**, 366.

² Sachs, A., Levine, V. E., and Fabian, A. A., *Arch. Int. Med.*, 1935, **55**, 227.

to the physiological level. In this type of therapy it is often advisable to diminish the red cell count even below the normal in order to postpone the rapid return of the polycythemic state. The drug used, phenylhydrazine hydrochloride, is a cumulative poison, so that its destructive effect upon the erythrocytes goes on after its discontinuance. A polycythemic individual therefore in the course of the treatment now prevailing may be converted to an anemic individual with a low red cell count. In periods of non-treatment the red cells have a tendency to increase to the polycythemic stage. The periods of treatment alternated by periods of non-treatment offer a chance to study the reciprocal relationship between blood iron and blood copper.

The second case is a white woman, 65 years of age. She was under treatment a short time before being referred to us for observation and further treatment. Her red cell count was well over 7,000,000, the blood volume was increased, her spleen was enlarged, she had vertigo, the characteristic facies and other findings of a true polycythemia vera.

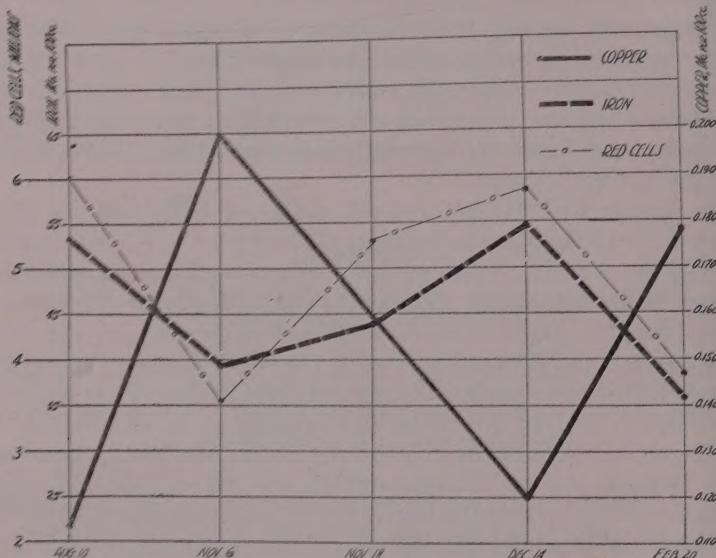
On August 10, 1935, the red cell count was 6,080,000, the blood iron was 53.16 and the blood copper 0.113 mg. or 113.5 micrograms per 100 cc., a figure below normal. She was placed on phenylhydrazine treatment, which was carried out irregularly and intermittently, since the patient showed a marked intolerance for the drug. Difficulty was experienced in finding the weekly dose of phenylhydrazine that would maintain the red cell count at the normal level. On November 6 her red cell count had dropped to 3,520,000 and her blood iron had gone down to 39.36 mg., while her blood copper had risen to 0.200 mg. or 200 micrograms per 100 cc. On November 18 after a period of non-treatment, the red cells went up to 5,376,000, the blood iron to 44 mg. per 100 cc. and the copper fell to 0.158 mg., or 158 micrograms per 100 cc. Treatment was temporarily discontinued.

On December 14 the red count was 5,760,000, the blood iron 54.79 mg. and the blood copper 0.120 mg. or 120 micrograms per 100 cc. Further treatment with phenylhydrazine being considered inadvisable due to the sensitivity of the patient to the drug, radiation with X-ray over the long bones was attempted for a short time in an effort to inhibit the bone-marrow in its production of red cells.

The patient disliked the X-ray treatments, and hence she did not receive sufficient radiation substantially to lower the red cell count. On December 30, 1935, venesection was performed and about 800 cc. of blood removed. It was now found that by decreasing the dose of phenylhydrazine, the patient could take the drug with greater toler-

ance. On February 20, 1936, the red cell count was 3,776,000, the blood iron 35.88 mg. per 100 cc. and the blood copper, 0.178 mg., or 178 micrograms per 100 cc. She is now on a weekly ration of phenylhydrazine and is progressing very well.

We have plotted graphs to show the hypoferronemia and the corresponding hypercupremia observed during treatment with phenylhydrazine and the increase in the red cells and in the blood iron during periods of non-treatment accompanied by a fall in blood copper. We have published elsewhere the graph accompanying the findings in the first case.² The graph accompanying the findings in the second case reported here is given below.



Fluctuations in Erythrocytes, Blood Iron, and Blood Copper in Female Patient, 65 years of age, suffering with Polycythemia Vera, in periods of treatment and non-treatment. Aug. 10, 1935, treatment with phenylhydrazine begun; Nov. 6, 1935, treatment stopped; Dec. 30, 1935, venesection performed.

Iron was determined by the Wong³ method and the results were checked with the dry ashing method recommended by us.⁵ The copper was determined by McFarlane's method.⁴ Slight modifications were introduced in both procedures.^{1, 2, 5}

Copper and iron are stored in the liver. It may be argued that

³ Wong, S. Y., *J. Biol. Chem.*, 1928, **77**, 409.

⁴ McFarlane, W. D., *Biochem. J.*, 1932, **26**, 1022.

⁵ Fabian, A. A., Sachs, A., and Levine, V. E., *Proc. Soc. Exp. BIOL. AND MED.*, 1935, **32**, 662.

phenylhydrazine damages the liver and renders it unable to retain copper. The copper that is forced to leave the liver mobilizes into the circulating fluid, and as a result an increase in blood copper ensues. If this reason holds true for the rise in blood copper, we are at a loss to understand why under the same circumstances the blood iron should fall considerably.

Iron and copper may be considered catalysts of oxidation. As a matter of fact the enzymes concerned in biologic oxidation, such as peroxidase and catalase, are organic complexes containing iron.⁶

Copper is a much more active catalyst than iron. Warburg⁷ cites Meyerhof, who observed that copper accelerated the oxidation of fructose to the extent of 140%, while iron only increased the oxidation by 70%. According to Voegtlín, Johnson and Rosenthal,⁸ the oxidation of glutathione is easily accomplished in the presence of copper, whereas iron fails in this reaction as a catalyst. Mawson⁹ has reported that ascorbic acid is relatively stable in glass distilled water, which is free from copper and iron. Copper, and to a lesser extent iron, and especially a mixture of these two elements, act as positive catalysts in the aerobic oxidation of ascorbic acid. Jones and Smedley-MacLean¹⁰ have shown that the oxidation of phenyl derivatives of fatty acids with hydrogen peroxide is greatly accelerated by the presence of copper. Cunningham¹¹ has observed that copper markedly accelerated the oxidation of dopa (1,3,4 dihydroxyphenyl alanine) by dopa oxidase. Even in the absence of this enzyme the oxidation of the reagent was to some extent catalyzed by copper alone.

Keil and Nelson¹² have reported what they term "a hitherto undescribed property of copper" in connection with carbohydrate metabolism. They showed that by oral administration of copper alone to experimental animals made anemic, they could bring the abnormal anemic glucose tolerance curve for the blood back to normal values even before any hemoglobin regeneration became evident. They assigned this phenomenon to the activity of copper with relation to the physiologic oxidative processes involving carbohydrate.

⁶ Kuhn, W., Hand, D. B., and Florkin, M., *Z. Physiol. Chem.*, 1931, **202**, 255.

⁷ Warburg, O., *Science*, 1925, **61**, 575.

⁸ Voegtlín, C., Johnson, J. M., and Rosenthal, S. M., *Pub. Health Rep.*, 1931, **46**, 2234.

⁹ Mawson, C. O., *Biochem. J.*, 1935, **29**, 569.

¹⁰ Jones, R. O., and Smedley-MacLean, I., *Biochem. J.*, 1935, **29**, 1877.

¹¹ Cunningham, I. J., *Biochem. J.*, 1931, **25**, 1267.

¹² Keil, H. L., and Nelson, V. E., *J. Biol. Chem.*, 1934, **106**, 343.

It seems that when the quantity of the catalyst, iron, falls below the normal, the biologic system attempts to compensate for the dangers of lessened oxidative activity by an increase in the content of the more vigorous and the more highly efficient catalyst, copper. The body needs little copper under normal conditions and the rise under abnormal conditions need not be very high, since a small quantity of this element goes a long way in the mechanism of catalysis.

In the event of hypoferronemia the observed rise in the copper content of the blood leads to the conclusion that the biologic organism mobilizes copper, which is poured into the blood from the store-houses. The copper thus mobilized not only acts in the capacity of a stimulator of hematopoietic activity, but also in the capacity of an emergency oxidative catalyst.

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Visualization of Preparalytic Lesions of Poliomyelitis by Intravital Staining.*

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The gradual descent of poliomyelic infection, after intranasal inoculation, through the olfactory tract, the basal ganglia, midbrain, pons and medulla to the spinal cord was demonstrated in experimental animals (monkeys) by Faber and Gebhardt,¹ using the method of recovering virus from bits of nervous tissue excised on successive days of the preparalytic period. Evidence was presented by Faber² that the infection in man may follow a similar pathway. Since this method of recovering virus permits only a rough localization, other means were sought to obtain a more precise definition. Evans and MacCurdy³ reported successful vital staining of poliomyelic lesions in monkeys, but failed to state the dye used or the period of infection when the animals were examined. McClellan and Goodpasture⁴ were able, by means of intravital staining with

* This study was supported by a grant from the Rockefeller Fluid Research Fund.

¹ Faber, H. K., and Gebhardt, L. P., *J. Exp. Med.*, 1933, **57**, 933.

² Faber, H. K., *Medicine*, 1933, **12**, 83.

³ Evans, H. M., and MacCurdy, J. T., *Berl. klin. Wochenschr.*, 1912, **49**, 1695.

⁴ McClellan, R. H., and Goodpasture, E. W., *J. Med. Res.*, 1924, **44**, 201.

trypan blue and subsequent clearing of the tissues, to visualize the lesions of another axon-conducted viral disease, herpetic encephalitis. Their method has been used in the present study of the sites and character of the lesions in the preparalytic stage of experimental poliomyelitis after intranasal inoculation carried out by the technique of Schultz and Gebhardt.^{5†}

Results in 3 rhesus monkeys are here reported: 1. a control; 2. an animal killed 3 days after inoculation; 3. an animal killed 6 days after inoculation, before paralysis or weakness occurred.

In the control, which was given no inoculation with virus but received trypan blue in the same manner as the inoculated animals, there is no visible staining of the nervous tissues proper. The dura, velum interpositum and choroid plexuses are deeply stained, while the leptomeninges are faintly stained. The smaller arteries and veins are inconspicuous and there is no evidence of capillary dilatation.

In the 3-day animal, the appearance of the tissues is similar except in the diencephalon, where the smaller blood vessels appear dilated, giving this area a streaked and dotted appearance; and just ventral to the main body of the thalamus and dorsal to the corpus mamillare, somewhat lateral to the midline, there is an oval area about 3 by 5 mm. in diameter which is stained blue. On inspection with a hand lens, minute bluish specks can be seen in the thalamus itself.

In the 6-day animal, in which no poliomyelic signs or symptoms had been noted, the appearance of the nervous tissues presents a striking contrast with that of the control and of the 3-day animal. Within the brainstem are many large rounded and banded areas of deep blue, in which the corpora mammillaria, the nuclei of the thalamus, the midbrain (red nucleus), the ventral portion of the pons and several portions of the medulla are conspicuous. Much of the striate body escapes staining but some is found in the head of the caudate nucleus and in the putamen. The olfactory bulbs are completely unstained, as is the cerebellum. In the cortex scattered small areas of fairly intense staining are found in the precentral region, in the hippocampal gyrus and in the parolfactory area. The gray matter of the spinal cord also shows good blue staining, particularly the anterior horns and the posteromedial portion.

⁵ Schultz, E. W., and Gebhardt, L. P., Proc. Soc. EXP. BIOL. AND MED., 1933, **30**, 1010.

† The monkeys were inoculated for us by Mr. L. P. Gebhardt at the laboratories of the Department of Bacteriology of Stanford University through the kindness of the Head of the Department, Professor E. W. Schultz.

Under the microscope the localizations of the stain are found to be of two sorts. In the brain stem, the macroscopically visible blue color is due to an accumulation of dye in the walls (especially the endothelial nuclei), and to some small extent in the plasma, of the capillaries. The number of these which are open is enormously greater than in the control. No perivascular, pericellular or meningeal invasion with wandering cells has occurred, except in the medulla where some small pericellular infiltrates are visible. The same phenomenon is found in the spinal cord. In addition, some, but not more than half, of the anterior and posterior horn cells are found to be stained blue, usually with the dye collected in granules at or near the periphery and in some cases within the nucleus. The distribution of the stained cells is curiously uneven: it is neither symmetrical nor uniform at different levels; moreover stained and unstained cells are to be found side by side in the same section. Although no nerve-cell staining is seen outside the spinal cord, the microscopic appearance of some of the cells in the brainstem, particularly the medulla, is not entirely normal. The experiments indicate that the earliest secondary reaction in the central nervous system to invasion by virus is simple hyperemia, beginning about the third day and reaching a high degree by the sixth day of the incubationary period. This reaction precedes general invasion of the pericellular, perivascular and meningeal spaces by wandering cells of the microglial, leucocytic and lymphocytic series. It may, moreover, be completely absent, as in the olfactory bulbs, where virus first appears and is known to be continuously present in high concentration throughout the period of invasion.

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Effect of Low Levels of Fluorine Intake on Bones and Teeth.

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A large number of investigations with different species are in agreement that the toxicity of fluorine, as measured by growth and certain physical and chemical changes in the bones, varies with the form of fluorine fed. A recent study by Smith and Leverton¹ with rats, while substantiating this conclusion as regards levels necessary

¹ Smith, M. C., and Leverton, R. M., *Ind. Eng. Chem.*, 1934, **26**, 791.

to retard growth or to cause death, shows that various compounds fed at a much lower fluorine level, namely 14 ppm., were all equally effective in causing enamel pigment changes in the incisors of rats. These observations raise the question as to why the results should be different at different levels and as to the significance of the pigment changes in terms of bone and tooth structure. We therefore undertook a study at the lowest levels used by Smith and Leverton, which included bone and teeth analyses for fluorine as well as observations of pigment changes. For this study, sodium fluoride, generally reported among the most toxic sources, and bone meal, a natural product containing a relatively small amount of fluorine in a complex insoluble form, were selected.

In view of the recent reports of the appreciable and variable occurrence of fluorine in common foods, it was deemed desirable to use a basal diet of known fluorine content. After considerable study the Sherman diet used by Smith and Leverton, consisting of two-thirds wheat and one-third whole milk with 1.3% of salt, was selected as the lowest one found. This diet, as made up by us, contained approximately 3 ppm. of fluorine. The bone meal used contained 380 ppm. Our fluorine analyses of the foods, bone and teeth were made by ashing with calcium oxide as a fixative at 500°C., distillation with perchloric acid at 135°C., as outlined by Willard and Winter² and by the determination of fluorine in the distillate according to the method of Sanchis.³ The incisors were examined at frequent intervals for the pigment changes and graded according to the degree of lightening of the pigment observed.

Growing rats which had been fed the basal diet from weaning until placed on experiment were used. One group continued to receive the basal diet alone, while a second received the same diet plus sodium fluoride and a third, bone meal, the supplements being added at levels which would provide the fluorine intake under study. In order to keep the calcium and phosphorus relations the same, appropriate amounts of calcium oxide and monocalcium phosphate were introduced into the basal diet and the sodium fluoride diet to balance the minerals added by the bone meal. These calcium and phosphorus salts were especially prepared to be as low as possible in fluorine and according to our analyses were sufficiently low that they did not add materially to the fluorine content of the rations in which they were introduced.

In the first experiment rats 53 days of age were fed the 3 diets

² Willard, H. H., and Winter, O. B., *Ind. Eng. Chem., Anal. Ed.*, 1933, **5**, 7.

³ Sanchis, J. M., *Ind. Eng. Chem., Anal. Ed.*, 1934, **6**, 134.

in which the fluorine sources were included to provide an added fluorine level of 14 ppm. After 62 days on the diets the animals were killed and their leg bones dissected for analyses. Food intake records were kept and it was found that without restricting the intake for any group they were substantially alike on the 3 diets. The results of the teeth and bone examinations are given in Table I.

TABLE I.
Comparison of Sodium Fluoride and Bone Meal at a Fluorine Level of 14 ppm.

| Diet | Age when killed, days | Wt. when killed, gm. | F in bones, dry basis, ppm. | Tooth pigment changes |
|--|-----------------------|----------------------|-----------------------------|-----------------------|
| Control | 115 | 300 | 91 | 0 |
| | | 280 | 141 | 0 |
| | | 212 | 156 | 0 |
| | | 288 | 95 | 0 |
| | | 292 | 112 | 0 |
| Aver. | | | 119 | |
| Control plus NaF (14 ppm. F) | 115 | 284 | 370 | lightening |
| | | 334 | 400 | , |
| | | 292 | 430 | , |
| Aver. | | | 400 | |
| Control plus Bone Meal (14 ppm. F) | 115 | 280 | 347 | , |
| | | 322 | 361 | , |
| | | 312 | 400 | , |
| Aver. | | | 369 | |

These data show the entire absence of pigment changes in the teeth of the control animals, in contrast to the distinct and similar changes on the diets containing sodium fluoride and bone meal. Particularly striking are the marked and similar increases in the fluorine content of the bones where the supplements were added, as compared to the results with the basal diet.

A second experiment was carried out in which the fluorine sources were added at a level of 8 and 12 ppm. The animals were started at 60 days of age. Some of each group were killed for bone examination after 56 days on experiment and the others at 168 days, in order to observe any progressive changes with age. The results are shown in Table II. While the number of animals for a given treatment are few, the data are sufficiently uniform to make certain conclusions clear.

It is noted that for each diet and level of feeding there is a marked increase in the fluorine content of the bones after 168 days as compared with the content at 56 days. This presumably reflects an increase which normally occurs with age. The data for both 56 and 168 days, however, clearly show that a fluorine addendum as low as 8 ppm. results in a marked increase in the fluorine content of the bones, compared to the content for animals of the same age on

TABLE II.

Comparison of Sodium Fluoride and Bone Meal at Fluorine Levels of 8 and 12 ppm.

| Diets | Days on diet | Age when killed, days | Wt. when killed, gm. | F in bones, dry basis, ppm. | Lightening of tooth pigment |
|----------------------------------|--------------|-----------------------|----------------------|-----------------------------|-----------------------------|
| No. 4 | | | | | |
| Control | 56 | 118 | 212 | 94 | 0 |
| | | | 236 | 85 | 0 |
| | | | 187 | 96 | 0 |
| Aver. | | | | 92 | |
| No. 4 | | | | | |
| Control | 168 | 230 | 322 | 176 | 0 |
| | | | 320 | 135 | 0 |
| | | | 237 | 150 | 0 |
| Aver. | | | | 154 | |
| No. 5 | | | | | |
| Control plus NaF (8 ppm.) | 56 | 118 | 148 | 226 | 0 |
| | | | 246 | 257 | slight |
| | | | 213 | 296 | 0 |
| | | | 210 | 282 | very slight |
| Aver. | | | | 265 | |
| No. 5 | | | | | |
| Control plus NaF (8 ppm.) | 168 | 230 | 350 | 374 | " " |
| | | | 348 | 364 | " " |
| | | | | 369 | |
| Aver. | | | | | |
| No. 6 | | | | | |
| Control plus Bone meal (8 ppm.) | 56 | 118 | 244 | 248 | lightening |
| | | | 232 | 292 | very slight |
| | | | 185 | 267 | " " |
| Aver. | | | | 269 | |
| No. 6 | | | | | |
| Control plus Bone meal (8 ppm.) | 168 | 230 | 213 | 368 | " " |
| | | | 376 | 352 | " " |
| | | | 228 | 376 | lightening |
| Aver. | | | | 365 | |
| No. 7 | | | | | |
| Control plus NaF (12 ppm.) | 56 | 118 | 220 | 257 | very slight |
| | | | 212 | 235 | lightening |
| | | | 170 | 386 | slight |
| | | | 208 | 350 | lightening |
| Aver. | | | | 307 | |
| No. 7 | | | | | |
| Control plus NaF (12 ppm.) | 168 | 230 | 388 | 657 | lightening |
| | | | 248 | 456 | very slight |
| | | | | 556 | |
| Aver. | | | | | |
| No. 8 | | | | | |
| Control plus Bone meal (12 ppm.) | 56 | 118 | 213 | 345 | " " |
| | | | 166 | 369 | " " |
| | | | 206 | 323 | slight |
| Aver. | | | | 346 | |

the basal diet. The response to bone meal and to sodium fluoride appears to be identical, in agreement with the results in Table I. The limited data for the level of 12 ppm. also present a similar picture and it is noted that for a given period on experiment the fluorine content is uniformly higher than at the level of 8 ppm.

The data for pigment changes in the teeth are in agreement with

the bone analyses in showing an effect at the level of 8 ppm. The variable data reveal no differences between the 2 sources of fluorine, nor for the different lengths of time on experiment. The teeth of the animals from the groups killed at 56 days were combined and analyzed for fluorine with the following results:

| | Fluorine, ppm. |
|------------------------------|----------------|
| Controls | 81 |
| Sodium fluoride, 8 ppm..... | 228 |
| Bone meal, 8 ppm..... | 220 |
| Sodium fluoride, 12 ppm..... | 246 |
| Bone meal, 12 ppm..... | 276 |

These data confirm the results of the bone analyses in showing the distinct effect of the small fluorine additions, in revealing no differences between the two supplements, and in showing a greater effect at the higher level.

It cannot be stated, without further study, whether the pigment changes and increases in fluorine content obtained in these experiments represent a definite structural injury. The diet fed our stock rats has regularly contained one per cent of bone meal. In a recent examination of our entire colony of mature animals most of them showed a slight lightening of the enamel, the others showing no detectable change. Analyses of the bones of animals approximately 500 days of age gave an average fluorine value of 335 ppm.

Summary. The data here reported show clearly that fluorine additions of 8 to 14 ppm. to a basal diet containing 3 ppm. results in growing rats in increases in the fluorine content of the bones and teeth, which are marked and roughly proportional to the level fed. They show sodium fluoride and bone meal to be equally effective in causing these changes. They suggest that analysis for fluorine is a more sensitive measure than the enamel pigment changes which are also noted at these low levels. The data indicate that the fluorine content of the bones and teeth increases with age during growth.

8837 C

Purification of Tetanic Toxin.

MONROE D. EATON. (Introduced by S. Bayne-Jones.)

From the Department of Bacteriology, Yale University School of Medicine.

The methods used in the purification of tetanic toxin were based on the general principles outlined in a previous paper on the purifi-

cation of diphtherial toxin.¹ The crude toxins were prepared in meat-infusion containing 2% of Bacto-peptone, and 1% dextrose. Cultures of *Cl. tetani* were grown 14 days under vaseline and filtered. Lots 4 and 5 were about one year old, and lots 6 and 8 were fresh when the purification was started.

Purification. The toxic preparations should be kept in a refrigerator except when reagents are added or when being centrifuged. The optimal proportions of reagents vary somewhat with different lots of toxin. These variations have been indicated in the directions for the process given below. Amounts are for the purification of one liter of crude toxin.

1. To one liter of crude toxin 100 to 125 cc. of 10% solution of ferric ammonium sulphate are added gradually. The pH is kept between 5.5 and 6.0 by the addition of normal sodium hydroxide. The resulting precipitate will dissolve in an excess of the ferric salt below pH 5.5. After settling for a few hours the precipitate is collected and washed with distilled water. The toxin is then eluted with 400 cc. of 2.5% sodium citrate at a pH of 7.5 to 8.0 for 3 days or until most of the precipitate has dissolved. The insoluble residue is centrifuged down and the pH of the supernatant adjusted to 7.0.

2. Four hundred cc. of the eluted toxin are diluted to one liter and the calculated concentration of sodium citrate adjusted to 1.5 to 2.5%. Three hundred to 450 cc. of 5% cadmium chloride are added until no further precipitation occurs and the pH is near 5.8. After 2 hours the precipitate is centrifuged, washed, and emulsified in about 300 cc. of 4% sodium chloride. Most of the toxin is dissolved out of the precipitate if the pH of the suspension is adjusted to 6.8 with a few drops of normal hydrochloric acid, but very little toxin dissolves above pH 7.5. After standing over night at a pH of 6.8 the insoluble residue is centrifuged down. The clear yellow supernatant contains the toxin.

3. To 300 cc. of this supernatant are added 15 cc. of 20% sodium citrate and then 2.5% solution of neutral lead acetate until the solution is quite opalescent. (About 50 cc. of lead acetate.) The precipitate gradually increases in amount and after settling over night is centrifuged down and discarded. If not too much lead acetate has been added the toxin will remain in the supernatant. This step is included here because it is advantageous in removing inactive protein and colored substances from certain preparations, but it may be omitted.

¹ Eaton, M. D., *J. Bact.*, 1936, **31**, 347, 367.

4. On the solutions of toxin resulting from steps 2 or 3 precipitation with cadmium chloride is repeated by adding an equal volume of 5% cadmium chloride and adjusting the pH to 7.8 with 5% barium hydroxide. After an hour the precipitate is centrifuged down and eluted with 2% sodium bicarbonate adjusted to a pH of 7.8 by the addition of phosphoric acid. After standing over night the residue is centrifuged down. The bicarbonate solutions of toxin must be kept in tightly stoppered containers to prevent loss of carbon dioxide. If the solution becomes strongly alkaline the toxin will be destroyed. The clear almost colorless elution of the cadmium precipitate contains the purified toxin.

Some of the preparations were dialyzed in cellophane against running water for 48 hours to remove salts and residual peptones. A partial precipitation of the toxin may occur during dialysis. Sodium bicarbonate solutions of toxin should be buffered at pH 7.0 with anhydrous phosphate before dialysis.

Results of Purification. At least 50% of the total M.L.D. of tetanic toxin should be recovered after each of the 4 steps of the purificatory process just described. The resultant yields of purified toxin are 10 to 30%.

Representative results of the procedure are presented in Table I. The M.L.D. was determined by subcutaneous injection at the groin. Ascending tetanus resulted from all active preparations. Since some of the purified toxins seem to be partially inactivated by dilution, 0.1 cc. of the appropriate dilution was injected in preference to 1.0 cc. of a 10-fold greater dilution. Like crude tetanic

TABLE I.
Purification of Tetanic Toxin Expressed in Terms of Nitrogen per M.L.D.

| Crude toxin No. | Purificatory process* | M.L.D. per cc. for mice | M.L.D. per cc. for guinea pigs | Mg. N† per cc. | Mg. x 10 ⁻⁶ N/M.L.D. mice | Mg. x 10 ⁻⁶ N/M.L.D. guinea pigs |
|-----------------------|--------------------------|-------------------------------|--------------------------------------|-------------------|--|---|
| 4 | crude | 500 | — | 2.16 | 4,300 | — |
| 4 | 1, 2 | 2,500 | — | 0.305 | 122 | — |
| 3 | crude | 3,000 | 1,500 | 2.90 | 960 | 1,930 |
| 5 | 1, 2 | 10,000 | — | 0.260 | 26 | — |
| 3 | 1, 2, 3, 4 | 8,000 | 4,000 | 0.053 | 6.6 | 13 |
| 5 | 1, 2, 3, 4, D | 2,000 | 1,500 | 0.027 | 12.0 | 18 |
| 6 | crude | 5,000 | 2,000 | 2.32 | 460 | 1,160 |
| 6 | 1, 2 | 2,500 | 1,000 | 0.044 | 17.0 | 44 |
| 6 | 1, 2, 4 | 10,000 | 5,000 | 0.046 | 4.6 | 9 |
| 8 | crude | 4,000 | — | 2.65 | 650 | — |
| 8 | 1, 2, D | 2,000 | — | 0.022 | 11.0 | — |
| 8 | 1, 2, 3, 4 | — | 1,500 | 0.018 | — | 12 |

* The steps of the procedure used in purification are designated by numbers corresponding to those in the text. D = dialysis.

† Nitrogen determined by Pregl micro Kjeldahl method. In the last 2 columns nitrogen per M.L.D. is expressed in millionths of a milligram.

toxin, the purified toxins are 5 to 10 times as toxic for guinea pigs, per gram of body weight, as for mice.

The process separates tetanic toxin from 99.0 to 99.5% of the nitrogenous impurities. The dried organic residue from the purified dialyzed toxin contains about 12% of nitrogen. Twenty-five to 50% of the total nitrogen in purified toxin is precipitated by 5% trichloracetic acid as protein. At least part of the remaining nitrogen is in proteoses or peptones. The purified preparations when sufficiently concentrated give positive biuret, Millon, xanthoproteic, diazo, and sulphur reactions. In some the Molisch test is positive, in others, doubtful.

For a 500 gm. guinea pig the M.L.D. of the purest preparations contains 9 to 18 millionths of a milligram of nitrogen. This represents a fatal dose of 0.00015 to 0.00030 mg. of dried organic material per kilo of guinea pig. The lethal dose of purified diphtherial toxin previously reported¹ was 0.00040 to 0.00060 mg. per kilo.

The preparation of highly active dried tetanic toxin by fractionation with ammonium sulphate and dialysis has been reported by London and Aristovsky² and by Brieger and Cohn.^{3, 4} The methods used by these authors have not been as successful in our hands as those described in the present paper.

Although the purified preparations are obviously still a mixture of substances, it seems likely that the purification of more powerful crude toxins than those used in the present work by improved methods would produce even more active purified toxins.

¹ London, E. S., and Aristovsky, V. M., *Compt. rend. Soc. de Biol.*, 1917, **90**, 756.

² Brieger, L., and Cohn, G., *Z. f. Hyg. u. Inf.*, 1893, **15**, 1.

³ For a review of earlier purification experiments see: Kolle, W., Kraus, R., and Uhlenhuth, P., *Handbuch der Pathogenen Mikroorganismen*, Dritte Auflage, 1929, **2**, 391, 531; 1928, **4**, 1035.

8838 C

Influence of Acetylsalicylic Acid (Aspirin) on Urinary Excretion of Ascorbic Acid.

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From the Department of Nutrition, Iowa Child Welfare Research Station, State University of Iowa.

During a recent study of the vitamin C needs of children which included determinations of the daily urinary excretions of ascorbic acid, one child developed a slight upper respiratory infection which interfered with night rest. He was given on 2 successive nights 2.5 grains of aspirin; and water ingestion was forced during 2 days. Since his food intake remained normal and his temperature was only slightly elevated, the experiment was not discontinued as is usual when a child who is under observation develops a cold. Analysis of the urine following aspirin administration showed an increased output of ascorbic acid. This was quite out of line with our general findings to the effect that following physiologic adjustment, at a given level of ingestion the daily excretions of ascorbic acid of the children under observation were surprisingly constant during the 10-day period of study (Everson and Daniels¹). Was the increased excretion of ascorbic acid due to conditions producing the elevated temperature, the effect of the acetylsalicylic acid, or to a washing-out process following the larger water intake? That infections may deplete the stores of vitamin C has been suggested by Dry,² by Harde, Rothstein and Ratish,³ and by Harde and Benjamin.⁴ On the other hand, Giardina⁵ has shown that sodium salicylate will precipitate scurvy and death in guinea pigs receiving diets containing an insufficient amount of the antiscorbutic vitamin in a shorter time than in pigs on similar diets with no sodium salicylate.

To obtain further data regarding the effect of aspirin on vitamin C metabolism in the human organism, the 3 children between 4 and 6

¹ Everson, G. J., and Daniels, A. L., *J. Nutrition*, 1936, **12**, 15.

² Dry, T. J., *Arch. Int. Med.*, 1933, **51**, 679.

³ Harde, E., Rothstein, I. A., and Ratish, H. D., *PROC. SOC. EXP. BIOL. AND MED.*, 1935, **32**, 1088.

⁴ Harde, E., and Benjamin, H. R., *PROC. SOC. EXP. BIOL. AND MED.*, 1935, **32**, 651.

⁵ Giardina, J. J., and Ets, H. N., Report given at meeting of Am. Soc. Pharm. and Exp. Ther., Washington, D. C., 1936.

years of age who were under observation were given constant weighed diets during which period the ascorbic acid intakes and excretions were determined daily. Orange juice, the chief source of the ascorbic acid, was given in 2 equal portions, one in the morning and one in the afternoon. At the end of the third day, each child was given 2.5 grains of aspirin, the dose usually prescribed for children of this age. After a period of 3 days, 2.5 grains were again given. Rectal temperatures were taken 3 times daily. In order to ascertain if other urinary constituents were affected, creatinine, nitrogen and phosphorus determinations were made with the daily excretions. During the study, one of these children (F.V., 4/3) also developed a slight cold and for 1 day carried a slightly elevated temperature (99°-102°).

The methods used for preparing and storing the food and collecting the urine were the same as those of the previous report (Everson and Daniels¹). The urinary ascorbic acid of the first child to receive the aspirin (J. E., 1/13-23) was determined by means of the 2:6 dichlorophenolindophenol method (Birch, Harris and Ray⁶). Subsequently, the urinary ascorbic acid was determined both by the 2:6 dichlorophenolindophenol and the phospho-18-tungstic acid method (Medes⁷) in order to rule out the possible interfering phenols and thiol compounds. The ascorbic acid of the food was determined by the dichlorophenolindophenol method previously described.

Aspirin, as such or combined with urine, reacts neither with the 2:6 dichlorophenolindophenol indicator nor with the phospho-18-tungstic acid reagent. The findings, therefore, (Table I) would seem to indicate that acetylsalicylic acid increases the urinary excretion of vitamin C. In this respect the action apparently is similar to that of ether (Zilva,⁸ Bowman and Muntwyler⁹). Whether this increased output is the result of a specific effect on vitamin C metabolism or an increased kidney permeability is not shown. Both nitrogen and phosphorus excretions were slightly increased, whereas the creatinine eliminations were unaffected with the exception of the period of elevated temperature, when it was increased. There was no constant relation between urine volume and vitamin C elimination. Cushny¹⁰ states that there is a 10-12% increase in nitrogen

⁶ Birch, T. W., Harris, L. J., and Ray, S. N., *Biochem. J.*, 1933, **27**, 590.

⁷ Medes, G., *Biochem. J.*, 1935, **29**, 2251.

⁸ Zilva, S. S., *Biochem. J.*, 1935, **29**, 1612; 1935, **29**, 2366.

⁹ Bowman, D. E., and Muntwyler, E., *PROC. SOC. EXP. BIOL. AND MED.*, 1935, **33**, 437.

¹⁰ Cushny's *Pharmacology and Therapeutics*, 9th Ed., p. 527. Lea and Febiger, Philadelphia, 1928.

ASPIRIN AND ASCORBIC ACID EXCRETION

PART II. Influence of Acetylsalicylic Acid (Aspirin) on Urinary Excretion of Ascorbic Acid, Creatinine, Phosphorus and Nitrogen.

| | | | | | | | |
|-------|------------------------------|-------|------|------|-------|------|------|
| G.E.† | 3/31-4/1 | 99.5 | 860 | 29.2 | 275 | .486 | 6.38 |
| | 4/1-2 | 99.5 | 1005 | 30.0 | 32.0 | .578 | 6.77 |
| | 4/2-3 | 99.5 | 905 | 31.5 | 29.0 | .558 | 6.77 |
| | 2.5 grains aspirin—7:15 P.M. | | | | | | |
| | 4/3-4 | 99.5 | 1180 | 62.4 | 61.2 | .604 | 7.05 |
| | 4/4-5 | 99.5 | 925 | 39.4 | 42.4 | .614 | 7.04 |
| | 4/5-6 | 99.5 | 970 | 39.9 | 37.5 | .638 | 7.08 |
| | 2.5 grains aspirin—7:15 P.M. | | | | | | |
| | 4/6-7 | 99.5 | 930 | 55.0 | 55.3 | .656 | 7.43 |
| | 4/7-8‡ | 99.5 | 810 | 41.4 | 41.0 | .245 | 6.14 |
| F.V.† | 3/31-4/1 | 102.4 | 1310 | 30.5 | 29.5 | .568 | 7.28 |
| | 4/1-2 | 102.4 | 1210 | 36.5 | 34.3 | .587 | 7.64 |
| | 4/2-3 | 102.4 | 1200 | 39.9 | 37.3 | .512 | 7.47 |
| | 2.5 grains aspirin—7:15 P.M. | | | | | | |
| | 4/3-4 | 102.4 | 1690 | 96.0 | 106.6 | .740 | 7.33 |
| | 4/4-5 | 102.4 | 930 | 46.7 | 46.3 | .304 | 7.88 |
| | 4/5-6 | 102.4 | 1300 | 33.6 | 34.2 | .304 | 8.00 |
| | 2.5 grains aspirin—7:15 P.M. | | | | | | |
| | 4/6-7 | 102.4 | 1280 | — | 304 | .642 | 8.60 |
| | 4/7-8‡ | 102.4 | 700 | 24.5 | 26.1 | 269 | 7.92 |

* Daily urine collections 6 A.M. to 6 P.M.

† Daily urine collections 6 P.M. to 6 P.M.

‡ Twenty-four hour excretion estimated on twelve hour collection.

§ Titration with 2:6 dichlorophenolindophenol.
|| Phospho-18-tungstic acid colorimetric method.

and sulphur excretion, and 30-45% increase in uric acid excretion following aspirin medication, suggesting an effect on metabolism. Fine and Chace¹¹ observed an increase in the uric acid excretion with a compensating decrease in blood uric acid following the ingestion of sodium salicylates, which was attributed to an increase in kidney permeability. In line with these findings are those of Zilva⁸ to the effect that following ether anesthesia the tissues of the animals studied gave no indication of a decreased vitamin C fixation, since the ascorbic acid content of these tissues was comparable to those of normal animals. On the other hand, ether anesthesia appears to affect muscle metabolism since not only is nitrogen¹² and phosphorus excretion increased, but the blood phosphorus also is increased (Bolliger¹³), the result according to Stehl and Bourne¹⁴ of a withdrawal from the muscles.

Further studies of conditions affecting ascorbic acid elimination may explain the frequently observed association of scurvy and rheumatoid arthritis (Rinehart¹⁵) in cases where sodium salicylate has been the choice of medication.

8839 C

Thyrotropic Hormone in Non-Pituitary Tissue.

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Sturm and Schöning¹ recently published data which indicated that certain organs, notably the ovaries and adrenal cortices contained large quantities of thyroid-stimulating substance. They reported from 11,000 to 56,000 guinea pig units (Junkmann and Schoeller) per 100 gm. desiccated ovaries and up to 36,000 units per 100 gm. desiccated adrenal cortex. While these non-hypophyseal extracts were said to exert atypical thyrotropic effects in that increasing doses were not followed by correspondingly increased thyroid hyperplasia and that the minimally effective doses were inconstant, the physiological significance of thyrotropic sub-

¹¹ Fine, M. S., and Chace, A. F., *J. Biol. Chem.*, 1915, **21**, 371.

¹² Hawk, P. B., *J. Biol. Chem.*, 1908, **4**, 321.

¹³ Bolliger, A., *J. Biol. Chem.*, 1926, **60**, 721.

¹⁴ Stehle, B. L., and Bourne, W., *J. Biol. Chem.*, 1924, **60**, 17.

¹⁵ Rinehart, J. F., *Ann. Int. Med.*, 1935, **9**, 586, 671.

¹ Sturm, A., and Schöning, W., *Endocrin.*, 1935, **16**, 1.

stances in such tissues was considered of great theoretical and practical importance.

Ballif and Gherseovici² repeated the work of Sturm and Schöning using ovaries as well as other non-hypophyseal tissues. They found that Loeser extracts (used by Sturm and Schöning) of ovaries were inactive in total doses equivalent to 30 mg. desiccated gland while parallel anterior lobe extracts caused typical histological changes in guinea pig thyroids in doses corresponding to far less tissue.

We have repeated these experiments using 4 types of extracts. (1) A crude Loeser 1.25% NH₄OH extract³ of acetone-desiccated beef, sheep and hog ovaries; this was neutralized to pH 7.0. (2) A crude dilute acetic acid extract of beef, sheep and hog ovaries, also neutralized. (3) A powder made from beef ovaries according to the Loeser method; this type of preparation was used by Sturm and Schöning. (4) A powder prepared from beef and hog ovaries by a method which when applied to beef or hog anterior lobes yields uniformly, material of 8 to 10 guinea pig units per mg.

The Junkmann and Schoeller technic was varied slightly by injecting 180-200 gm. guinea pigs on 4 successive days, instead of 3, and removing the thyroid glands on the 6th day. Several regions of each gland were observed.

The maximal total amount of crude NH₃ (Loeser) or acetic acid extract injected was 4 cc., equivalent to 300 mg. of desiccated ovarian tissue. The largest dose of the Loeser acetone precipitate of beef ovaries was 30 mg., equivalent to 10 gm. of desiccated ovaries. Of our own preparation from beef and hog ovaries the largest amount injected was 40 mg., equivalent to 1.7 and 4.2 gm. of desiccated tissue respectively. At least 4 animals on each level were used. In no case did positive thyrotropic responses occur.

An explanation of the results of Sturm and Schöning cannot be found unless they overlooked the fact that in guinea pigs of 200 gm. weight, a histological picture of the thyroid described by Junkmann and Schoeller as indicating early or initial thyrotropic activity of pituitary extracts is seen in a fairly large portion of control groups. We have found, in fact, that $\frac{1}{4}$ of our control animals show +1 reactions on a scale in which +4 is a maximal reaction at least as judged by appearances in certain portions of a section. One out of 20 control guinea pigs have +2 thyroids. We find, therefore, that it is necessary in evaluating thyrotropic activity to require for a unit effect a +2 reaction in 3 of 4 animals on a given dosage level

² Ballif, L., et Gherseovici, I., *Compt. Rend. Soc. Biol.*, 1936, **121**, 1437.

³ Loeser, A., *Arch. f. exp. Path. u. Pharm.*, 1932, **166**, 693.

and a confirmatory +3 response on the next higher dosage group.

Conclusion. No anterior-pituitary-like thyrotropic activity could be demonstrated in beef, sheep or hog ovaries.

8840 C

Presence of Estrin in Rat Pregnancy Urine.*

F. E. D'AMOUR, D. FUNK AND M. B. GLENDENNING.

From the Research Laboratories, University of Denver.

Many efforts have been made to demonstrate the presence of estrin in the urine of pregnant rats, particularly because of the wide use of this animal in the study of the physiological effects of estrogenic substances. We have previously made several unsuccessful attempts. Since it has been learned that acid hydrolysis greatly increases the yield of estrin from human pregnancy urine it seemed worth while to repeat the experiment.

The urine was collected by placing towels in the cages and covering them with screening. Record was kept of the number of animals and the length of time spent in the cages; the results are

TABLE I.

| Material Injected | Rat/Days Urine Inj'd per Rat | No. Rats Inj'd | Results |
|---------------------|------------------------------|----------------|---------------------------------|
| Normal Female Urine | 70 | 2 | 1 Negative 1 Weakly Positive |
| " " " | 35 | 4 | All Negative |
| 1st Week Pregnancy | 37.5 | 1 | Positive |
| " " " | 26.25 | 4 | All Negative |
| 2nd " " | 49 | 1 | Positive |
| " " " | 37 | 1 | " |
| " " " | 26 | 2 | " |
| " " " | 24 | 2 | 1 Positive 1 Weakly Positive |
| " " " | 13 | 2 | Both Negative |
| 3rd " " | 60 | 1 | Positive |
| " " " | 52.5 | 1 | " |
| " " " | 30 | 2 | " |
| " " " | 27.5 | 2 | " |
| " " " | 24.5 | 3 | " |
| " " " | 15.5 | 4 | All Negative |
| Hydrolyzed Plac. | 48 Plac. per Rat | 1 | Negative |
| " " | 24 " " " " | 2 | " |
| Unhydrolyzed Plac. | 48 " " " " | 1 | " |
| " " | 24 " " " " | 2 | " |

* This investigation was aided, in part, by a grant from the National Research Council, Committee on Problems Related to Sex.

therefore expressed in rat-days of urine. The towels were extracted twice for 12 hours with alcohol, the alcohol removed by evaporation and the sludge hydrolyzed for 2 hours with 10% hydrochloric acid. Following hydrolysis, the material was extracted with ether in a continuous extractor for 12 hours. The ether solution was washed with sodium bicarbonate solution and placed in olive oil. The divided dosage method of Marrian was used for the assay.

It happened to be convenient to assay rat placentae for estrin at the same time. One hundred and ninety-two placentae from rats 21 days pregnant were finely ground and refluxed twice for 24 hours with ethyl alcohol. This extract was purified by treatment with petroleum ether and with acetone. It was then divided and one-half subjected to acid hydrolysis. The results of both experiments are shown in Table I.

Conclusion. Small, but demonstrable amounts of estrin were found in the urine of pregnant rats; no estrin could be demonstrated in rat placentae.

8841 P

Phagocytic Activity of Bone-Marrow Cells.

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W. L. Mallman.)

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In view of the fact that there is a vigorous opsonic response to the parenteral injection of antigenic fractions of Brucella cells, an unusual opportunity is offered for measuring the phagocytic activity of the various types of bone marrow cells collected directly from the marrow, or as they are found in the peripheral blood in myelogenous leukemia.

Studies have been made on the phagocytic activity of marrow cells aspirated from the cavity of the femur of normal guinea pigs, and those immunized to Brucella, and on marrow cells in the peripheral blood of one human case of chronic myelogenous leukemia.

The cells in the blood of the pigs were measured for their Brucella phagocytic activity *in vitro* prior to the collection and examination of marrow cells. The cells in the whole blood of normal pigs

showed no phagocytic activity. The segmented and stab neutrophile forms in the whole blood of the immunized pigs were found to ingest at least 50 or more bacteria; thus indicating the presence of immune opsonins in the serum for Brucella.

The phagocytic activity of marrow cells from normal and immunized guinea pigs was measured *in vitro* in the presence of both normal guinea pig serum and serum containing immune opsonins.

The phagocytic activity of the marrow cells in the peripheral blood of the case of myelogenous leukemia was examined in the presence of the patient's serum and in the presence of a serum obtained from another person possessing a high immune opsonin titer for Brucella. The mixtures of cells, serum and bacteria, were incubated in a water bath at 37°C. for 30 minutes before making spreads and staining.

The forms of marrow cells from guinea pigs that were examined were: megaloblasts, macro normoblasts, normoblasts, primitive myeloblasts, myeloblasts, premyelocytes, myelocytes, metamyelocytes, juvenile forms, stab forms, neutrophiles, filamented neutrophiles, eosinophiles and basophiles.

In the leukemia case, only cells of the myelogenous series were studied.

The results obtained follow: Normal guinea pig marrow cells + normal guinea pig serum + bacteria; no cellular ingestion of bacteria noted.

Normal guinea pig marrow cells + immune opsonin serum + bacteria: all filamented neutrophiles contained more than 50 bacteria; the stab form varied in its ingestion capacity: from 10 to more than 50 bacteria were seen in a cell. All other forms of the myelogenous series and the nucleated forms of the erythrocytic series showed no phagocytosis of the bacteria.

Immune guinea pig marrow cells + immune opsonin serum + bacteria: phagocytic picture similar to the one mentioned in the previous paragraph with the exception that an occasional juvenile form was found that had ingested from 1 to 10 bacteria.

Immune guinea pig marrow cells + normal guinea pig serum + bacteria: all filamented and stab neutrophile forms ingested bacteria in numbers varying from 10 to above 50; 10% of the juvenile form ingested less than 15 bacteria; all other forms of the myelogenous series and the nucleated forms of the erythrocytic series showed no phagocytosis.

The leukemic whole blood (0.8% sodium citrate) had a total cell count 200,000 per cu.m.; differential count on 100 cells gave myeloblasts 32, myelocytes 13, metamyelocytes 10, juveniles 10, stabs

23, filamented forms 8, lymphocytes 0, monocytes 1, eosinophiles 2, basophiles 1.

Leukemic whole blood + bacteria: no phagocytosis of bacteria seen.

Leukemic blood cells + serum of individual containing immune opsonins + bacteria: all filamented forms showed ingestion of more than 50 bacteria; the ingestion capacity of stab forms varied from zero to more than 50 bacteria per cell; their activity appeared to depend upon the age of the cell. None of the cell forms younger than the stab were observed to take up bacteria.

The inference to be drawn from these observations is that, in infections where mature neutrophiles are being rapidly destroyed, the bone marrow in an attempt to replace the cells destroyed, forces out into the peripheral blood a high percentage of young forms which play little if any part in the protection of the individual against the infection. It would appear, therefore, that a too rapid or continuous shift to the left in peripheral blood cells of the myelogenous series is not a desirable condition. Its continuance impairs one of the important defensive mechanisms of the body during infection.

8842 P

Effects of Synthetic Progesterone on Female Genital Tract of the Monkey.*

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The corpus luteum hormone corporin as obtained from the corpus luteum will produce premenstrual development of the endometria of castrated monkeys and traumatization of such uteri stimulates the formation of epithelial proliferations characteristic of normal implantation sites.^{1, 2} It is also known that oestrin will promote growth of the uterus, cornification of the vagina, development of the sex-skin, and metaplasia of the cervical glands.^{3, 4}

* Aided by a grant from the National Research Council Committee on Problems of Sex.

¹ Hisaw, F. L., *Am. J. Obst. Gyn.*, 1935, **29**, 638.

² Engle, E. T., Smith, P. E., and Shelesnyak, M. C., *Am. J. Obst. Gyn.*, 1935, **29**, 787.

³ Overholser, M. D., and Allen, E., *Surg. Gyn. Obst.*, 1935, **60**, 124.

⁴ Engle, E. T., and Smith, P. E., *Anat. Rec.*, 1935, **61**, 471.

When effective doses of oestrin and corporin are administered simultaneously oestrin does not prevent the action of corporin on the endometrium nor does it prevent epithelial proliferation following trauma. Corporin, however, minimizes the effects of oestrin on the sex-skin and vagina, and prevents metaplasia of the cervical glands.^{5, 6}

Experiments have been conducted to determine whether or not the synthetic corpus luteum hormone, progesterone, possesses the above physiological properties known for the natural substance. The material used was prepared synthetically from Stigmasterol by the method described by Butenandt and furnished by the Schering Corporation, Bloomfield, N. J.

The type of experiment employed is illustrated by the following protocol: Monkey Al. 54, adolescent, body weight 4075 gm., was given pituitary extracts resulting in marked ovarian development without luteinization. The animal was castrated and given 100 RU of oestrin daily for 15 days, followed by a combination treatment of 100 RU of oestrin plus 4 international units of progesterone daily for 10 days. The uterus was traumatized on the eighth day of oestrin-progesterone treatment and the animal was killed at the termination of the experiment.

Histological examination of the genital tract of this animal showed a premenstrual endometrium approaching secretory exhaustion, marked epithelial proliferations surrounding the traumatic areas, an absence of squamous metaplasia of the cervical glands, and a reduced oestrin effect on the vaginal mucosa as indicated by relatively few mitoses, subepithelial leucocytic infiltrations, and the absence of Dierk's layer over extensive areas. These results agree with those previously reported for experiments in which combinations of oestrin and corporin were used,^{5, 6} and indicate that synthetic progesterone has the same physiological properties as the progestational hormone extracted from corpus luteum tissue.

⁵ Hisaw, F. L., *Anat. Rec.*, 1935, **64**, 54, sup. No. 1.

⁶ Hisaw, F. L., and Lindrum, E. C., *Endocrinology*, 1936, **20**, 228.

8843 P

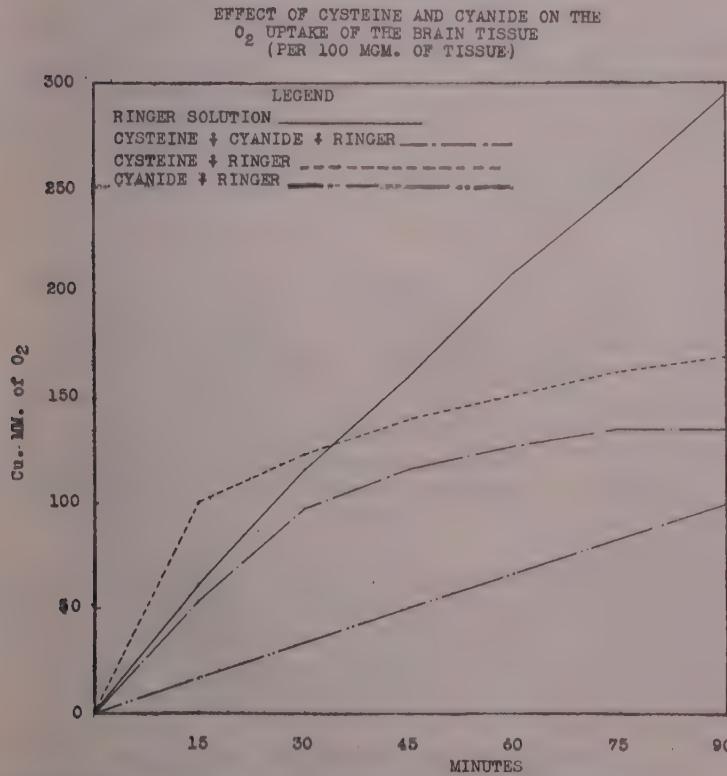
Effect of Cysteine on the Metabolism of the Isolated Brain Tissue.

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HIMWICH.

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We are reporting experiments elsewhere on the effects of cysteine and cystine on the metabolism of rats (Goldfarb, Fazekas, Himwich¹). The present communication is a report of further experiments on isolated brain tissue. The observations were limited to the effects of cysteine, since cystine was soluble in solutions

FIGURE 1.



¹ Goldfarb, W., Fazekas, J. F., and Himwich, H. E., (to appear in the *Am. J. Physiol.*).

too alkaline or acid to support respiration. In all experiments the tissue was suspended in a phosphate medium buffered at pH 7.4 with lactate as a substrate. The tissues were inserted in the Warburg respirometer and one-half hour elapsed, the time required for equilibration, before observations were begun.

The data disclose that the O_2 consumption of the brain tissue treated with cysteine exhibits a diphasic response. During the first 30 to 45 minutes there is a marked stimulation which is followed by a profound depression in the later periods. Cysteine, moreover, retains an early stimulatory effect on O_2 consumption despite the presence of cyanide.

In order to obtain further information as to the character of the increased O_2 consumption, a group of observations was made on the R.Q. after the addition of cysteine hydrochloride in a concentration of 0.01 M. In most instances the R.Q. of unwashed, minced brain tissue is approximately 0.9, (Himwich, Fazekas, Barker, Hurlburt²) and in these studies the addition of cysteine resulted in a depression of the quotient. In 8 experiments they were found to be 0.50, 0.65, 0.65, 0.54, 0.59, 0.52, 0.30, and 0.83. The presence of the sulphydryl group had obviously caused a consumption of O_2 without the simultaneous production of CO_2 .

8844 C

Relation of Anterior and Posterior Lobe of the Hypophysis to Insulin Sensitivity in the Rat.*

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The discovery of Houssay and Magenta,¹ since repeatedly confirmed by other investigators, that the hypophysectomized animal becomes extremely sensitive to insulin, led to attempts to determine what part of the pituitary body was involved. The observation

* Himwich, H. E., Fazekas, J. F., Barker, S. B., Hurlburt, M. H., *Am. J. Physiol.*, 1934, **110**, 348.

* Aid by the Presnell Fund for the study of the metabolic relations of the anterior hypophysis contributed by Robert R. Presnell, Frank Tuttle, Arthur Stebbins and Mrs. Gordon Kahn of Los Angeles.

¹ Houssay, B. A., and Magenta, M. A., *Rev. Asm. Med. Argentina*, 1924, **37**, 389; *Comp. Rend. Soc. de Biol.*, 1925, **92**, 822.

of Burn² that extracts of the posterior lobe protected normal fasting rabbits against the convulsive action of insulin was confirmed by Geiling, Campbell and Ishikawa³ in experiments on hypophysectomized dogs. The latter authors also reported that removal of the anterior lobe did not render the dog hypersensitive to insulin, while various operative procedures designed to interfere with posterior lobe secretion had this effect. They concluded that the antagonistic action of the hypophysis to insulin was attributable to the activity of the posterior lobe. Houssay and Potik,⁴ on the other hand, found that extirpation of the anterior lobe made toads hypersensitive to insulin and that the daily implantations of one anterior lobe under the skin afforded protection, while similar implantations of posterior lobe had no protective action. This pointed to the removal of the anterior lobe as the cause of the hypersensitivity to insulin.

In connection with anterior lobe removal in the dog it should be pointed out that the anatomical relationships of the canine hypophysis make the complete removal of the anterior lobe extremely difficult, if not impossible, because a portion of the anterior lobe envelopes the dorsal part of the posterior lobe. There is still another factor which has to be considered when the temporal approach is used in the dog as has been done by Geiling and associates, namely, that of brain injury. As shown by Chaikoff, Reichert and collaborators,⁵ retraction of the temporal lobe for 2 minutes, so as to expose the pituitary to view but without actually touching it, resulted in hypersensitivity to insulin which persisted in some cases for 3 months.

In view of the conflicting results just enumerated, it seemed desirable to extend this work to an animal form in which the anatomical relationship of the hypophysis is such that it permits the independent removal of either of the two main portions of the hypophysis. Such a condition is offered by the rat. The rat presents another advantage. The sub-diaphragmatic approach to the gland, first described by Smith, makes it possible to remove either lobe unaccompanied by brain injury. It must be admitted, however, that removal of the posterior lobe does involve slight injury to the anterior lobe. Removal of the anterior lobe, on the other hand,

² Burn, J. H., *J. Physiol.*, 1923, **57**, 318.

³ Geiling, E. M. K., Campbell, D., and Ishikawa, Y., *J. Pharm. Exp. Therap.*, 1927, **81**, 247.

⁴ Houssay, B. A., and Potik, D., *Comp. Rend. Soc. Biol.*, 1929, **101**, 940.

⁵ Chaikoff, I. L., Beichert, F. L., Larson, P. S., and Mathes, M. E., *Am. J. Physiol.*, 1935, **112**, 493.

can be accomplished without injury to the pars intermedia or pars nervosa. The attachment of the stalk to the brain also remains intact. The histological evidence of the success of such separation will be considered elsewhere.

The experimental material included 164 female rats. Of this total, 105 were completely hypophysectomized; 13 had the posterior lobe and variable portions (in most cases one-half) of the anterior lobe removed; 14 had posterior lobe and a very small fragment of the anterior lobe excised; and 22 had the anterior lobe alone removed. In the last group, the pars nervosa and the intermediate lobe, including their attachment to the brain, were left intact. Brief summaries of the different operative groups are shown in Table I.

TABLE I.

| Type of Operation | No. of Animals | Insulin (units/kilo) | No.* Reacting | No. not† Reacting |
|-----------------------------|----------------|----------------------|---------------|-------------------|
| Complete Hypophysectomy | 30 | 1 | 25 | 5 |
| | 63 | 0.5 | 53 | 10 |
| | 12 | 0.25 | 8 | 4 |
| Incomplete Hypophysectomy‡ | 13 | 1 | 2 | 11 |
| Anterior lobe only removed§ | 7 | 2 | 7 | 0 |
| | 11 | 1 | 11 | 0 |
| | 2 | 0.5 | 2 | 0 |
| | 2 | 0.25 | 2 | 0 |
| Posterior lobe removed | 14 | 3 | 1 | 13 |

* Convulsion--injected with glucose.

† Spontaneous recovery.

‡ Posterior lobe and approximately one-half of anterior lobe removed.

§ Anterior lobe alone removed. Pars nervosa and pars intermedia intact.

|| Posterior lobe and a very small fragment of anterior lobe removed.

The hypophysectomized rat is a fragile animal and it seemed inadvisable to secure blood samples by repeated heart punctures. Therefore, in testing its reaction to insulin, the minimum amount of insulin necessary to cause convulsions was used as a measure of insulin sensitivity. Although animals subjected to incomplete hypophysectomy involving the removal of the posterior lobe and one-half or more of the anterior lobe, do show a somewhat greater reaction to small doses of insulin than do normal animals, they are nevertheless much less sensitive than completely hypophysectomized animals. If, however, the operation for removal of the posterior lobe is accompanied by only very slight injury to the anterior lobe, the response of such animals to insulin compares very favorably with that of normal controls. It will be seen that of the 14 animals tested with 3 units of insulin per kilo body weight, only one de-

veloped convulsions; in the remaining 13 animals no disturbance such as tremors or weakness could be detected. On the other hand, the behavior of rats after the anterior lobe only was removed was in no respect different from that shown by the completely hypophysectomized rat. Of the 22 animals tested, 7 succumbed after injection of 2 units per kilo and the intraperitoneal injection of glucose proved of no avail. Eleven of the animals which had received 1 unit of insulin per kilo developed repeated convulsions even after the administration of glucose. The remaining 4 animals, 2 receiving 0.5 units and the other 0.25 units per kilo, also showed convulsions, their death being prevented only by the immediate injection of glucose.

TABLE II.
Blood Sugar After Insulin Injection.*

| | Blood sugar in mg. % | | | | | |
|---|----------------------|---------|-------|---------|-------|---------|
| | Initial | 0.5 hr. | 1 hr. | 1.5 hr. | 2 hr. | 2.5 hr. |
| Average of 5 normal rats (8-15 hours off food) | 113 | 98 | 100 | 111 | 110 | 105 |
| Average of 6 rats after removal of anterior lobe (3 weeks post-operative) (8-15 hours off food) | 65 | 34 | 15 | 16 | 15 | Died |

*0.25 units per kilo were injected intraperitoneally.

Six animals with the anterior lobe removed were anesthetized with amyta and blood was drawn from the jugular vein. The blood sugar values of these animals are shown in Table II. Although the posterior lobe was intact in these animals, the blood sugar dropped to the convulsive level one hour after the injection of only 0.25 units of insulin per kilo. The response of normal animals to a similar dose of insulin is included for comparison. Control experiments, using amyta alone, showed that this anesthetic, *per se*, was not responsible for the blood sugar changes.

Summary. Removal of the posterior lobe of the hypophysis with only slight injury to the anterior lobe does not increase the sensitivity of rats to the convulsive action of insulin. Removal of the anterior lobe only, leaving the posterior lobe intact, results in a marked increase in insulin sensitivity equal to that observed after complete hypophysectomy.

Increase in Production of Ethyl Alcohol by Yeast Treated with Ultra Violet Energy.

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Observations regarding the effect of ultra violet light upon the fermenting power of yeast have been made by various authors. Owen and Morley¹ report an increase in activity of yeast after administration of appropriate dosages, while Tanner and Byerly² demonstrated an adverse effect. The first noted authors seem convinced that irradiation of the medium by means of ultra violet energy before introduction of yeast results later in enhanced growth of the microorganism, but Tanner and Byerly were unable to confirm this finding.

The object of our series of experiments was to determine whether exposure of yeast to ultra violet light is attended by subsequent increase in formation of ethyl alcohol.

For our purposes, we have utilized equipment set up as follows: The source of ultra violet was a quartz tube of approximately 24 inches length and one centimeter external diameter, of the type known ordinarily as "Cold Quartz" which operates with emission of very little heat and with much energy in the region of 2500-2800 Å. Placed outside of this quartz electrode, was an ordinary glass tube. Materials to be treated with the ultra light thus could pass between the outer wall of the quartz tube and the inner surface of that made of glass. The system was set up in a vertical position and fluids to be treated entered at the bottom and were withdrawn at the top. A variety of glass tubes of different diameters enclosing the inner quartz electrode permitted alteration of the thickness of layer of the fluid to be treated. Proper mixing during the period of treatment was brought about by intermittent surges of air introduced likewise at the lower end of the vertically placed apparatus.

For these experiments we have used purified brewers yeast. The crude material which contained both Torula and certain Acetobacters as contaminants, was plated on Sabouraud agar. From these plates were picked 7 good sized colonies of yeast which were all fished to a flask of sterile beer wort and allowed to ferment as a

¹ Owen, W. L., and Morley, R. L., *Cent. f. Bakt. u. Parasitenk.*, 1933, 2nd Abt. 88, 273.

² Tanner, F. W., and Byerly, J. R., *Arch. Mikrobiol.*, 1934, 5, 349.

composite culture of known yeast. Six different tests were made with this material and incorporated in this series were varying periods of exposure to the ultra violet radiation. Only one result will be included here, but the others were similar in outcome.

A flask containing 300 cc. of beer wort which had been sterilized in the autoclave was inoculated with the culture of pure yeast and then was incubated for 36 hours at 37°C. After thorough shaking, 150 cc. of this culture then were removed for treatment with the ultra violet. Details of the radiation were as follows: Thickness of the layer at time of treatment, 1.15 cm. Period of treatment per cc., 1.4 seconds. After such exposure, 125 cc. of the yeast culture then were added to 1600 cc. of beer wort which had been sterilized previously by intermittent steaming. 125 cc. of the untreated culture were placed in a similar amount of wort for control. Incubation then proceeded at 28°C. In each experiment, there were removed from the fermenting preparations samples for alcohol determination at specified times. The alcohol computations were made immediately according to the procedure of the official methods of the Agricultural Chemists. Specific gravity also was read with correction for temperature. The periods of observation extended from 2 to 5 weeks. No counts of culture were made since yeasts clump with the result that plate determinations are of little value. Direct counts do not allow discrimination between live and dead cells.

When a pure culture of yeast in beer wort is exposed to the effect of ultra violet energy as demonstrated in Table I, the initial effect

TABLE I
The Specific Gravity Readings and Production of Ethyl Alcohol of Pure Yeast Culture in Beer Wort After Treatment with Ultra Violet Radiation. Control Untreated.

| Days | Specific gravity | | % ethyl alcohol | |
|------|------------------|---------|-----------------|---------|
| | Treated | Control | Treated | Control |
| 1 | .9975 | .9959 | 0.50 | 1.60 |
| 3 | .9953 | .9943 | 2.00 | 2.70 |
| 5 | .9944 | .9938 | 2.60 | 3.05 |
| 7 | .9944 | .9940 | 2.60 | 2.90 |
| 9 | .9928 | .9939 | 3.75 | 2.95 |
| 11 | .9935 | .9935 | 3.25 | 3.25 |
| 14 | .9933 | .9933 | 3.40 | 3.40 |
| 16 | .9934 | .9936 | 3.30 | 3.15 |
| 18 | .9931 | .9935 | 3.50 | 3.25 |
| 21 | .9927 | .9931 | 3.80 | 3.50 |
| 23 | .9932 | .9933 | 3.45 | 3.40 |
| 25 | .9932 | .9935 | 3.45 | 3.25 |
| 28 | .9930 | .9933 | 3.60 | 3.40 |
| 30 | .9933 | .9939 | 3.40 | 2.95 |
| 32 | .9937 | .9945 | 3.10 | 2.55 |

is to depress ability to form ethyl alcohol. In turn this phase is followed by increased fermenting activity after some days and this increase in production of alcohol is maintained thereafter. Additional experiments showed that over-intense exposure weakened the yeast so that fermentation induced by it thereafter was depressed. Very short exposures on the other hand, produce no evident effect whatever.

This increase in production of ethyl alcohol is due to the influence of the ultra violet energy upon the yeast itself and is not brought about by treatment of the wort. This was demonstrated by irradiating the beer wort under conditions identical to those already outlined. When such wort then was inoculated with untreated yeast, there was no appreciable difference between the amount of alcohol produced here and that formed by similar amounts of normal yeast in untreated wort.

8846 P

Effects on Blood Pressure of Injection of Kidney Extracts of Dogs with Renal Hypertension.

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A rise in blood pressure may be produced in dogs either by partial obstruction of the renal arteries¹ or by ligation of the ureters.² Since the latter procedure often causes a marked decline in the renal blood flow,³ it seems likely that the hypertension is in both instances related in some way to ischemia of the kidneys. It was shown by Tigerstedt and Bergman⁴ that saline extracts of the kidney of rabbits produced a sustained rise in blood pressure when injected into other rabbits. The object of our experiments was to determine whether extracts prepared from ischemic kidneys, removed from dogs with hypertension, had a greater pressor effect than similar extracts of normal kidneys.

The freshly removed kidneys were chopped up with scissors and

¹ Goldblatt, H., Lynch, J., Hanzal, R. F., and Summerville, W. W., *J. Exp. Med.*, 1934, **59**, 347.

² Harrison, T. R., Mason, M. F., Resnik, H., and Rainey, J., *Trans. Assn. Am. Phys.*. In press.

³ Levy, S. E., Mason, M. F., Harrison, T. R., and Blalock, A. In press.

⁴ Tigerstedt, R., and Bergman, P. G., *Skand. Arch. Physiol.*, 1898, **8**, 223.

ground with carborundum and 0.9% salt solution. The suspension so obtained was centrifuged and the supernatant fluid was kept in the ice box until used. Amounts corresponding to 5 gm. of kidney tissue were administered intravenously to *normal unanesthetized dogs*. Changes in blood pressure were measured by the cuff described by Ferris and Hynes,⁵ the passage of the pulse wave being determined by palpation of the dorsal artery of the foot.⁶

The results which have been obtained are summarized in Table I. Extracts of the normal kidneys frequently caused a marked preliminary decline in blood pressure, followed in a few minutes by a gradual rise which persisted for 30 minutes or longer. The degree of rise varied from zero to 60 mm. of mercury above the control values. Extracts of the kidneys of dogs with renal hypertension usually caused less preliminary decline and a more marked secondary rise in blood pressure. The time of onset of the peak of the blood pressure curve varied markedly in the different experiments, but tended to appear somewhat earlier when the extracts of the ischemic kidneys were injected.

In a number of experiments a comparison was made between the effects of extracts of the 2 kidneys of a dog made hypertensive either by partial obstruction of one renal artery or by unilateral ureteral ligation. Here again the extracts of the ischemic kidney caused less preliminary decline and more marked rise in blood pres-

TABLE I.

Effects on Blood Pressure of Unanesthetized Dogs of Injection of Extracts of the Kidneys of Normal Dogs and of Dogs with Renal Hypertension.

| Injected Saline Extracts of Kidney of | No. of Exp. | Decline in Blood Pressure: No. Observations | Max. Rise in Blood Pressure, mm. of Hg | | | Aver. Time of Max. Effect: Min. after Injection |
|---|----------------|---|--|---------|-------|--|
| | | | Lowest | Highest | Aver. | |
| Normal dogs | 12 | 6 | 0 | 75 | 27 | 29 |
| Hypertensive Dogs: | | | | | | |
| Partial obstruction of both renal arteries | 7 | 2 | 32 | 100 | 60 | 19 |
| Hypertensive Dogs: | | | | | | |
| Both ureters ligated | 4 | 0 | 0 | 132 | 47 | 8 |
| Hypertensive Dog: | | | | | | |
| Partial obstruction of one renal artery | | | | | | |
| Normal kidney | 4 | 1 | 0 | 30 | 14 | 26 |
| Ischemic kidney | 5 | 1 | 14 | 68 | 42 | 30 |
| Hypertensive Dog: | | | | | | |
| Ligation of one ureter | | | | | | |
| Normal kidney | 7 | 5 | 23 | 61 | 36 | 19 |
| Ischemic kidney | 8 | 0 | 34 | 71 | 49 | 14 |

⁵ Ferris, H. W., and Hynes, J. F., *J. Lab. and Clin. Med.*, 1930-31, **16**, 597.

⁶ Mason, M. F., Resnik, H., Minot, A., Rainey, J., Pilcher, C., and Harrison, T. R., *Arch. Int. Med.* In press.

sure than did the extracts of the opposite normal kidney of the same animal. (Table I.)

A few observations have been made on the effect of extracts of human kidney. It appears that the extracts of the kidney of certain patients with essential hypertension may have an increased pressor effect but this is not yet clearly established.

Our findings, which have been confirmed concurrently by Friedman and Prinzmetal,⁷ indicate that a relationship exists between experimental renal hypertension and the production in ischemic renal tissue of an increased amount of some pressor substance. Whether the latter is actually the cause of the rise in blood pressure is not yet certain. The findings are also compatible with the idea of a diminution in the rate of formation of a depressor substance in the ischemic kidney as a factor in the production of renal hypertension. Definite conclusions cannot be drawn until more is known concerning the chemical nature of the pressor and depressor agents. Attempts at separation and purification of these substances are now being made.

8847 C

Metabolic Activities of *Escherichia coli* in a Synthetic Medium.

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Recent studies by Martin,¹ Mooney and Winslow,² and Clifton^{3, 4, 5} on the metabolic activities of bacteria indicate that the rate of metabolic activity per cell varies widely at various phases of the growth-cycle. The maximal oxygen-consumption, carbon dioxide-production or ferricyanide-reduction per cell per unit-time was noted near the end of the lag-period of growth and could be only in part explained by increased cellular size during the same period of growth. The metabolic activities per unit-volume of the cultures, as measured by the above indices, reached maximal values

⁷ Friedman, B., and Prinzmetal, M. Personal communication.

¹ Martin, D. S., *J. Gen. Physiol.*, 1932, **13**, 691.

² Mooney, G., and Winslow, C.-E. A., *J. Bact.*, 1935, **30**, 427.

³ Clifton, C. E., Cleary, J. P., and Beard, P. J., *J. Bact.*, 1934, **28**, 541.

⁴ Clifton, C. E., *Proc. Soc. EXP. BIOL. AND MED.*, 1936, **34**, 291.

⁵ Clifton, C. E., *J. Bact.*, 1936. In press.

near the end of the logarithmic period of growth and then decreased rapidly as the age of the cultures increased. Peptone or other complex media were employed in these studies.

The present paper reports the influence of the concentration of lactic acid (sodium salt) on the oxygen-consumption, carbon dioxide-production and growth of *Escherichia coli* (K-12) in an inorganic medium of the following composition: NH_4Cl , 5.0 gm.; Na_2SO_4 , 2.0 gm.; MgSO_4 , 0.1 gm.; K_2HPO_4 , 3.0 gm.; KH_2PO_4 , 1.0 gm.; FeCl_3 (0.5% solution), 0.5 ml.; CaCl_2 (0.5% solution), 0.5 ml.; distilled water to make 1000 ml.

Oxygen-consumption was measured by the usual Warburg technic and carbon dioxide-production by the technic described by Walker.⁶ All tests were carried out at 37.5°C.

The influence of the concentration of lactic acid on the growth of *Esch. coli*, as measured with the aid of a photoelectric turbidimeter (Clifton⁷), is illustrated in Fig. 1, A. Concentrations of sodium lactate greater than 0.1 N markedly inhibited the growth of this organism.

The influence of 4 different initial concentrations of lactic acid

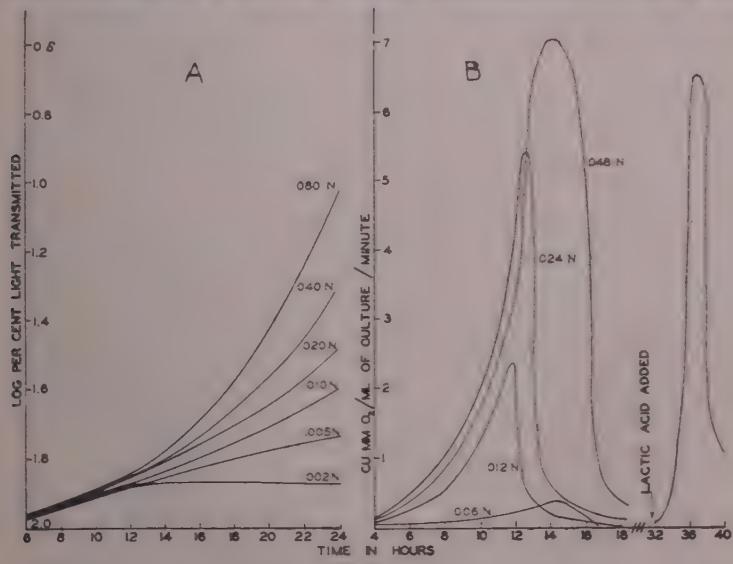


FIG. 1.

Influence of initial sodium lactate-concentration on the growth of *Esch. coli* (A) and on the oxygen-consumption of cultures of *Esch. coli* (B).

⁶ Walker, H. H., *J. Bact.*, 1932, **24**, 169.

⁷ Clifton, C. E., Mueller, Elizabeth, and Rogers, W., *J. Immunol.*, 1935, **29**, 377.

on the oxygen-consumption of *Esch. coli* is illustrated in Fig. 1; B, in which cubic millimeters of oxygen consumed per minute per millimeter of the cultures is plotted against time in hours. The typical change in oxygen-consumption following the addition of sufficient 2.0 N sodium lactate to make the cultures approximately 0.05 N with respect to the lactate is shown by the right-hand curve in Fig. 1, B. The lactate was added when the cultures were 31 hours old.

The influence of 4 different initial concentrations of lactic acid on the carbon dioxide-production and growth (viable count by dilution and plating) of *Esch. coli* is illustrated in Fig. 2, together with the changes in concentration of lactic acid during growth of the organisms. Lactic acid was determined by the method described by Friedemann and Kendall,⁸ the values reported being only approximate since duplicate determinations often varied by as much as $\pm 5\%$.

The rates of oxygen-consumption and of carbon dioxide-production per cell per unit-time reached maximal values during the lag or early logarithmic period of growth in this synthetic medium and then decreased rapidly with increasing age of the cultures.

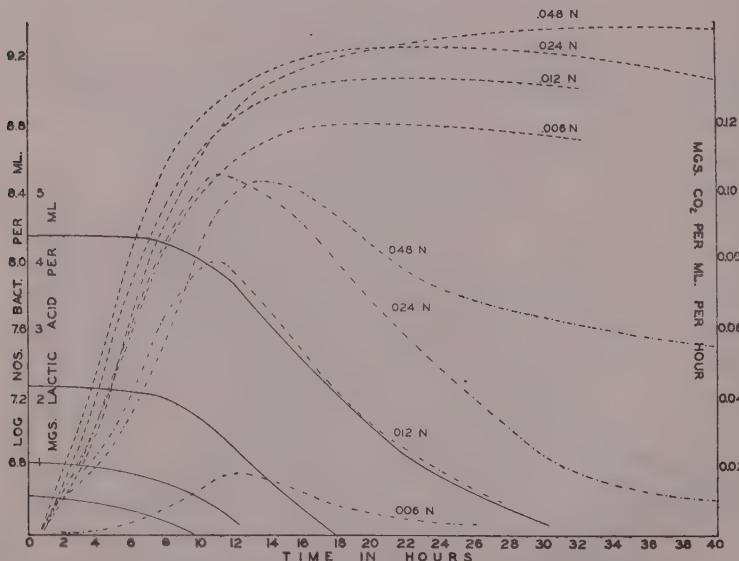


FIG. 2.

Influence of sodium lactate-concentration on growth (---) and on carbon dioxide-production (-----) of cultures of *Esch. coli*. Solid lines represent the change in concentration of lactic acid with time during growth of the organisms.

⁸ Friedemann, T. E., and Kendall, A. I., *J. Biol. Chem.*, 1929, **82**, 23.

The cells also appeared to be of maximal size during the early phases of growth. Typical values of the rate of carbon dioxide-production per cell per unit-time are presented in Table I.

TABLE I.

Results Illustrating the Changes in Lactic Acid-Concentration (mg. per ml.) and in Rates of Carbon Dioxide-Production (mg. $\times 10^{-10}$) per cell per hour in Cultures of *Esch. coli*.

| Age of culture in hours | Lactic acid | CO ₂ |
|-------------------------|-------------|-----------------|-------------|-----------------|-------------|-----------------|-------------|-----------------|
| 2-4 | 4.4 | 21.5 | 2.2 | 20.0 | 1.1 | 19.9 | .5 | — |
| 4-6 | 4.3 | 10.9 | 2.2 | 7.6 | 1.0 | 12.5 | .4 | .9 |
| 6-8 | 4.3 | 4.6 | 2.1 | 2.9 | 0.8 | 5.5 | .8 | .6 |
| 8-10 | 4.1 | 2.3 | 1.8 | 1.9 | 0.6 | 2.2 | .1 | .6 |
| 10-12 | 3.8 | 1.6 | 1.5 | 1.2 | 0.8 | 1.8 | — | .5 |
| 12-14 | 3.8 | 1.1 | 1.0 | 0.9 | 0.1 | 0.9 | — | .8 |
| 22-24 | 0.6 | 0.4 | — | 0.2 | — | 0.1 | — | — |

Maximal metabolic activity per unit-volume of the cultures was observed later in the growth-cycle, at a time which appears to be dependent upon the initial concentration of sodium lactate. Also, the total amount of carbon dioxide produced by the cultures appears to be directly proportional to the initial concentration of lactate. The pH of the cultures remained quite constant near 7.2.

The addition of lactic acid to 24-hour or older cultures resulted in an increased carbon dioxide-production, the rate changing with time in a manner similar to that reported for oxygen-consumption in Fig. 1.

The rate of oxygen-consumption of buffered suspensions of *Esch. coli* was almost independent of the concentration of lactic acid (concentration of bacteria constant) over the range of 0.4 to 20 mg. per ml., but rapidly decreased when the concentration of lactic acid was below or above the concentrations stated. The rate of oxygen-consumption was directly proportional to the number of bacteria with the exception that the rate per organism appeared to increase as the concentration of bacteria was decreased when the concentration of lactic acid was less than one mg. per ml.

These results suggest that as the numbers of bacteria increase in a culture the total amount of oxygen consumed, or of carbon dioxide produced, increases to a maximal value determined by concentrations of the reactants, the nature and size of the organisms and the physical and chemical influence of the environment on the cells. The decrease, increasing with age of the culture, in the rate of metabolic activity per cell may be interpreted on a basis of probability. As the number of bacteria increases the concentration-gradient of food-

stuffs between the cell and the environment decreases. Therefore, the probability of sufficient materials being available per cell per unit-time, to provide for the maximal possible requirement of the cells, decreases.

These results also lend further support to the hypothesis advanced by Cleary, Beard and Clifton⁹ that growth may be primarily controlled by this above probability-relationship, the growth-rate decreasing as the concentration per cell of materials essential for growth decreases in cultures of bacteria in which a relatively high population has been established. The maximal population developed under favorable conditions may also be primarily controlled by these concentration-relationships.

8848 P

Cysteine-Gelatin as a Differential Medium for *Salmonella pullorum* and *Salmonella gallinarum*.

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From the Department of Bacteriology, Yale University.

Differentiation of *Salmonella pullorum* from *Salmonella gallinarum* cannot be made by agglutination or agglutinin-absorption, because of a similar antigenic structure. This similarity and the fact that the 2 diseases produced in fowls are alike in some respects have caused certain European investigators^{1, 2, 3} to consider them as variants of the same species.

Mallman's⁴ work on the use of sodium-mucate agar, Jordan and Harmen's on tartrate-agar,⁵ and Naidu's⁶ and Nobrega's⁷ studies on differentiation by bacteriophage, are examples of investigations which supply further evidence that these organisms are separate entities. This report deals mainly with the use of cysteine-gelatin as another differential medium.

* Cleary, J. P., Beard, P. J., and Clifton, C. E., *J. Bact.*, 1935, **29**, 205.

¹ On sabbatical leave from the University of California.

² Miessner, H., Rept. Proc. 4th World's Poultry Cong., London, 1930, 428.

³ Wagener, K., Proc. 12th Internat. Vet. Cong., New York, 1934, **3**, 108.

⁴ Haupt, H., *Ergeb. der Hyg., Bakt., Immunit., u. Exper. Therapie*, 1935, **17**, 175.

⁵ Mallman, W. L., *PROC. SOC. EXP. BIOL. AND MED.*, 1931, **28**, 501.

⁶ Jordan, E. D., and Harmen, P. H., *J. Inf. Dis.*, 1928, **42**, 238.

⁷ Naidu, P. M. N., *Bul. Acad. Vet. France*, 1935, **8** (6), 306.

⁷ Nobrega, P., *Arch. do Instit. Biol. de S. Paulo, Brazil*, 1936, **6** (Artigo 9), 72.

The cysteine-gelatin was prepared according to a method used by Valley⁸ for the study of anaerobic growth. Enough Disco granular gelatin is added to the 0.15% cysteine-broth described by Valley⁹ to make a 12% solution. After dissolving the gelatin in a water-bath, the medium is tubed and sterilized at 15 pounds extra pressure for 15 minutes. Tubes of 12 mm. and of 16 mm. diameter were found to be equally satisfactory, when at least 5 cc. of medium were used. The cysteine-gelatin retained its potency for at least 2 months, when stored in the refrigerator and dehydration was prevented.

The action of 61 strains labeled *S. gallinarum*, 120 *S. pullorum*, and 11 *S. typhi*, was studied in this medium. Tartrate agar⁵ was inoculated with the same strains, for comparative purposes. A summary of these results is given in Table I. The tartrate-reactions support Mallman's findings. The results obtained with the 11 strains of *Salmonella typhi* are included also for record.

TABLE I.
Reactions in Cysteine-Gelatin and in Tartrate-Agar (37°C.).

| Bact. species | No. of strains | Reactions in Media | |
|---|----------------|--------------------|----------------|
| | | Cysteine-gelatin* | Tartrate-agar† |
| <i>S. gallinarum</i> (fowl origin) | 58 | + | + |
| <i>S. gallinarum</i> (Duisburg strains) | 3 | — | ± |
| <i>S. pullorum</i> | 120 | — | — to ± |
| <i>S. typhi</i> | 11 | ± | + |

* Cysteine. + = definite turbidity throughout the medium; ± = slight turbidity for the first 24 to 48 hours followed by a complete or partial clearing; — = no change.

† Tartrate. + = distinct acid reaction in the butt of the tube within 24 to 48 hours. ± = very slight acid reaction in butt in 24 hours, usually changing to alkaline in from 24 to 72 hours. Never markedly acid.

Fifty-eight of the *S. gallinarum* strains were from fowls. All gave a definitely positive reaction in cysteine-gelatin, in the form of a distinct yellowish-white turbidity when incubated at 37°C. for from 24 to 72 hours. The nature of this turbidity has not been definitely determined, but it is not due to profuse growth of the organisms. When stab-cultures of the same strain were incubated at 20°C., a zone of turbidity developed at the surface and along the line of puncture in from 48 to 72 hours. In shake-cultures a similar zone was found around each individual colony. The remaining 3 strains of "*S. gallinarum*" produced no perceptible clouding or precipitation. These were furnished through the courtesy of Dr. F.

⁸ Valley, George, Dept. of Bact., Yale Univ., Method unpublished, used by permission.

⁹ Valley, G., *J. Bact.*, 1929, 17, 12.

Kauffmann and were designated "Duisburg." Two were isolated from human feces, and the third from salad in the Duisburg outbreak of food-poisoning reported by Müller.¹⁰ These resembled *S. pullorum* in their deportment in cysteine-gelatin and in tartrate agar. Kauffmann¹¹ also noted minor differences between these and his fowl-strains.

The 120 strains of *S. pullorum*, all from fowls, produced no changes in the cysteine-gelatin, either when incubated at 37°C. or at 20°C. The reactions of the 11 strains of *S. typhi* (all of human origin) in cysteine-gelatin more nearly resembled those of *S. pullorum* than *S. gallinarum*. (See Table I.)

8849 P

Distribution of the Sub-groups of A and the M and N Agglutinogens Among the Blackfeet Indians.

G. ALBIN MATSON, PHILIP LEVINE AND H. F. SCHRADER.

From the Department of Biology, Montana State University, the Beth Israel Hospital, Newark, N. J., and the Office of Senior Physician, Blackfeet Agency, Browning, Mont.

The original observations of Matson and Schrader¹ on the Blackfeet and Blood tribes of American Indians revealed a distribution of the 4 blood groups vastly different from what was formerly believed to be characteristic for Indians. Group A was observed to have the same high preponderance (76.5%) among these tribes as group O has among other tribes of Indians.^{1, 2, 3, 4} These findings suggested that, contrary to former speculation concerning the origin of Indians, the "Blackfeet" did not separate from the rest of the human family before the A agglutinogen developed in the race.

In contrast to the characteristic differences of the two sorts of Indians with regard to the distribution of the 4 blood groups, subsequent work by us has shown that the Indians thus far studied, behave alike in having a high incidence of the M factor.²

¹⁰ Müller, R., *Münch. med. Wochensch.*, 1933, **80**, 1771.

¹¹ Kauffmann, F., *Zent. f. Bakteriol. I. Orig.*, 1934, **132**, 337.

¹ Matson, G. A., and Schrader, H. F., *PROC. SOC. EXP. BIOL. AND MED.*, 1933, **30**, 1380; *J. Immunol.*, 1933, **25**, 155.

² Levine, Philip, Matson, G. A., and Schrader, H. F., *PROC. SOC. EXP. BIOL. AND MED.*, 1935, **33**, 297.

³ Coca, A. F., and Deibert, O., *J. Immunol.*, 1923, **8**, 487.

⁴ Snyder, L. H., *Am. J. Phys. Anthrop.*, 1926, **9**, 233.

The present study which deals with the distribution of both M and N factors as well as the incidence of the sub-groups of group A, shows an exceedingly high percentage of the A¹ factor. The results are tabulated in Table I.

TABLE I.
Distribution of the Blood Groups, the sub-groups of A and the M and N Factors
Among the Blackfeet Indians.

| | | O | A | B | AB | A ¹ | A ² | MN | M | N |
|-------------------------|-----|-------|-------|-------|------|----------------|----------------|-------|-------|-------|
| Putative full-bloods | No. | 25 | 77 | 0 | 1 | 92 | 1 | 38 | 52 | 5 |
| | % | 24.27 | 74.76 | 0 | 0.97 | 99 | 1 | 40 | 54.74 | 5.26 |
| Whites | No. | 123 | 130 | 30 | 8 | | | 151 | 50 | 71 |
| | % | 42.27 | 44.67 | 10.31 | 2.75 | | | 55.52 | 18.38 | 26.10 |

These findings as to A and B agree with our previous observations of 103 putative full-blood Blackfeet Indians, a high proportion (77 individuals or 74.8%) belonging to group A.

Of 93 individuals* belonging to Group A, all but 1 (99%) belonged to sub-group A¹, as determined by tests with a serum of group B previously absorbed with cells A². This figure is to be contrasted with the corresponding values of 84% for whites and 55% for negroes in the studies of Landsteiner and Levine.⁵ In this connection, reference may be made to the 100% incidence of A¹ among 237 group A Hawaiians observed by Nigg.⁶ Incidentally, the distribution also of the 4 blood groups among these Hawaiians (36.5% group O, 60.8% group A, 2.2% group B, and 0.5% group AB) resembles that of the Blackfeet Indians.

In agreement with our previous findings among these Indians and with those of Landsteiner and Levine⁷ among Indians in Kansas reservations, the percentage distribution of the M factor was found to be high and that of N low. The gene frequencies of M and N for the Indians and the white race examined in this study, are 9.7 and 9.4 respectively.

Summary. Our previous findings with respect to the distribution of the 4 blood groups and the M factor among the Blackfeet Indians have been confirmed and a surprisingly high incidence of the A¹ sub-group was observed.

* Part of these were tested in a previous study.

⁵ Landsteiner, K., and Levine, Philip, *J. Immunol.*, 1930, **18**, 87.

⁶ Nigg, Clara, *J. Immunol.*, 1930, **19**, 93.

⁷ Landsteiner, K., and Levine, Philip, *J. Immunol.*, 1929, **16**, 123.

8850 C

Hydrogen-ion Concentration of the Bile of the Guinea Pig.*

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AND C. JELLEFF CARR.

From the Departments of Pharmacology and Gastroenterology, School of Medicine, University of Maryland.

Nichols¹ was the first to observe the comparatively greater degree of alkalinity of the bile of the guinea pig in his studies on the anti-septic action of the biles from various species. Using colorimetric methods Neilson and Meyer² corroborated the alkalinity of the gall bladder bile of the guinea pig. These workers observed the bile to be alkaline to litmus and phenolphthalein, but gave values of pH 7.7 and 7.8 to unexposed hepatic bile of the guinea pig, which they contend is slightly more alkaline than the bile from the gall bladder. During the course of experiments on the dissolution of gall stones, the authors have had occasion to determine the hydrogen-ion concentration of fresh bile from a large number of guinea pigs. The excessive alkalinity and uniformity in pH was deemed worthy of recording.

The bile was taken from the gall bladder with a syringe while the animal was under light ether anesthesia. The hydrogen-ion concentration was determined immediately without exposure using the Wilson³ type hydrogen electrode at $25^{\circ} \pm 0.5^{\circ}\text{C}$. Using the more acidic bile of the dog, experiments showed reasonably satisfactory agreement between the hydrogen electrode and the quinhydrone electrode, *i. e.*, ± 0.2 pH.

Determinations were made on 26 animals. The mean of the series is pH 8.89, the minimum value 8.66, the maximum value 9.14. In the series, the mean and the median are identical, the modal series is pH 8.85 to 8.95, $\sigma = 0.13$ pH and C.V. = 1.45.

Three composite samples of bile were analyzed by Douglas-Sauermann⁴ method. The average results showed: total solids 2.16%, ash 0.10%, mucin 0.51%, total lipid fraction 0.14%, bile acids determined as cholic and desoxycholic acids 0.78%. The analysis shows that the gall bladder bile of the guinea pig corresponds

* The expense of this investigation has been defrayed by the Julius Friedenwald Research Fund.

¹ Nichols, H. J., *J. Exp. Med.*, 1916, **24**, 497.

² Neilson, N. M., and Meyer, K. F., *J. Infect. Dis.*, 1921, **28**, 510.

³ Wilson, J., *Ind. Eng. Chem.*, 1925, **17**, 74.

⁴ Douglas-Sauermann, A. G., *Z. Physiol. Chem.*, 1935, **231**, 92.

generally to human bile in concentration of major constituents. Its hydroxyl-ion concentration, however, is much greater.

Summary. The pH of guinea pig's bile has been determined and an analysis of the principal constituents has been made.

8851 C

Inhibition of Oestrus and of the Vaginal Response to Oestrone by Testosterone.

J. M. ROBSON.* (Introduced by A. J. Clark.)

From the Department of Pharmacology, University of Edinburgh.

Mature mice showing regular oestrous cycles were injected with 0.1 mg. of testosterone in 0.1 cc. oil twice daily. (I am indebted to Messrs. Ciba, Limited, for the supply of testosterone.) During the period of injections extending over 2 weeks or more oestrus was abolished. Following cessation of injections oestrous cycles were reestablished. Injected mice were placed with males for a week; no mating was observed and no vaginal plugs were found. Normal cycles were continued in control mice injected with oil alone.

Mature ovariectomized mice were injected with oestrone in doses sufficient to produce cornification, together with testosterone. The simultaneous administration of testosterone inhibited the oestrous reaction of the vagina. The oestrogenic effect of 0.0001 mg. of oestrone (a dose which produces cornification in 50% of our mice) was inhibited by 0.4 mg. of testosterone.

These findings show that there may be a direct antagonism between the female and the male hormones in their actions on the vagina of the female mouse, and are the more remarkable in that testosterone in large doses has been shown to produce oestrogenic effects in the infantile rat (Butenandt and Kudzus¹).

Further experiments are now in progress to investigate quantitatively the relation between the actions of oestrone and testosterone on the vagina, and to determine whether other compounds with male hormone properties have effects similar to testosterone.

* Beit Memorial Research Fellow.

¹ Butenandt, A., and Kudzus, H., *Hoppe-Seyler's Zeit.*, 1935, **237**, 75.

Sensitivity of the Baby-Chick Comb to Male Sex Hormone.*

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EVANS.

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States Department of Agriculture.*

Of the various existing tests for the male sex hormone, that of the capon comb is generally recognized as being the most satisfactory. The cost of capons and their upkeep is a hindrance to wide use of this test. If baby chicks can be utilized to test for male hormone properties, the cost of such tests will be materially reduced.

The purpose of this investigation was to determine whether the comb of the baby chick is sufficiently sensitive to the action of male sex hormone to warrant the more extensive experiments necessary to establish that the baby chick may be used as a test object.†

As a preliminary experiment 3 groups of day-old, Single-comb White Leghorn chicks (7 chicks to the group) were injected subcutaneously with the male hormone product testosterone. A fourth group of 7 chicks was carried as controls. Group 1 received 25 gamma daily, and group 3 received 100 gamma. After 4 injections, 2 chicks of Group 1, 5 chicks of Group 2 and all those in Group 3 showed a definite increase in comb growth. Injections were continued on 3 chicks selected from Group 3. After 14 daily (except Sunday) injections these 3 chicks were photographed with their controls. Fig. 1 shows one of these chicks with its control.

In a second series of experiments, 117 other chicks were injected, some with testosterone and some with androsterone. Single-comb White Leghorns and Rhode Island Reds of both sexes were used. Their ages varied from 1 to 10 days of age, and injections of various amounts of material were made either in the base of the comb or in the breast muscle. Table I shows the results obtained. The control chicks in this series were injected with pure olive oil, except as otherwise designated in the table. The testosterone and androsterone were diluted with olive oil so that the smallest dose in any one experiment would be measurable with a fine syringe (0.01 cc.).

* The authors wish to express their thanks to Dr. Charles C. Haskell of the Ciba Co. of New York City for his kindness in furnishing the testosterone and androsterone used in these experiments.

† This work was undertaken in conjunction with a study of the physiology of reproduction which is being conducted with funds supplied under the Bankhead-Jones project.

TABLE I.
Weights of Combs of Chicks Injected with Testosterone (T) and Androsterone (A) and Controls.

| Breed | Days Age Injected | T No. | A Gamma | Injection site | Injected males No. | Aver. comb wt. Mg. | Control comb wt. males No. | Aver. comb wt. females No. | Control comb wt. females Mg. | |
|---------------------------|-------------------------|----------|------------|-------------------|--------------------------|-----------------------------|--|--|--|------|
| | | | | | | | | | | |
| Single Comb White Leghorn | 3 | 6 | 1.25 to 10 | Comb | 13 | 32.2 | 5 | 15.8 | 14 | 35.8 |
| | 6 | 6 | 0.62 to 5 | " | 10 | 43.6 | 3 | 33.7 | 6 | 39.5 |
| | 6 | 6 | 6.0-50 | " | 9 | 54.1 | | 7 | 59.3 | 2 |
| Rhode Island Reds | 6 | 6 | 0.62 to 5 | " | 9 | 18.6 | 2 | 18.0 | 6 | 17.5 |
| | 6 | 6 | 6.0-50 | " | 10 | 22.0 | | 6 | 6 | 24.2 |
| | | | | | | | | | | |
| Single Comb White Leghorn | 9 | 6 | 10 | " | 2 | 81.5 | 5* | 22.6 | 2 | 57.0 |
| | 9 | 6 | 10-40 | Breast | 15 | 55.5 | | | 5 | 34.8 |
| | 9 | 10 | 10 | Comb | 3 | 304.5 | 2* | 75.0 | — | — |

*Controls not injected with olive oil.



FIG. 1.

Chick 6028 received 10 injections (daily except Sunday) 1 B. U. testosterone in the base of the comb. The control was not injected.

The data in the table indicate that the female chicks were more sensitive than the male chicks, and that the White Leghorn chicks were more sensitive, at the ages used, than the Rhode Island Red chicks.

In most instances the chicks of the second series were injected from Monday to Saturday, inclusive, and sacrificed the following Monday. The one instance of 10 days of injections (See Fig. 1) and the one of 14 injections in the earlier experiment show results which indicate that the 8-day test may not fully utilize the sensitivity of the chick. However, the data are too few to be conclusive.

Chicks injected for 6 days in the base of the comb showed an average comb weight of a little over twice that of their controls, regardless of the amount of active material injected. It is therefore assumed that the smallest doses used, *e.g.*, 0.0 gamma of testosterone or 6.0 gamma of androsterone, produce a maximal response.

Two chicks that received 14 daily injections of 100 gamma of testosterone over a period of 10 days were kept under observation to note the subsequent comb growth after discontinuation of the injections. Thirty-five days after the injections were stopped, the combs of these chicks were still larger than those of their controls.

Obviously the young chick is quite sensitive to testosterone and androsterone. Further work is in progress to standardize it as a test object.

8853 C

Lipids of the Tooth Pulp.*

HAROLD C. HODGE. (Introduced by W. R. BLOOR.)

From the Department of Biochemistry and Pharmacology, School of Medicine and Dentistry, University of Rochester.

Tooth pulps were collected by cracking teeth. Fibrous, calcified and putrescent pulps were discarded. For human teeth, the sample of pulps weighed 0.43 gm. Micro-lipid analyses were carried out using the Bloor oxidative procedures. The total lipids were 0.91% (moist weight), phospholipids 0.70%, and cholesterol 0.11%. For cow teeth, 214 incisor pulps weighing 33.72 gm. were collected. After lipid extraction, saponification of the residue gave 0.16% lipids unextracted. The total ether-soluble lipids (0.85% of moist weight) contained 50% unsaponifiable matter; of the saponifiable fraction, the fatty acids composed 76.6%, iodine number—72.3. Using the Twitchell procedure, 79.9% liquid fatty acids and 3.0% solid fatty acids were found.

8854 P

An Electromagnetic Flowmeter. Principle of the Method and its Application to Bloodflow Measurements.

A. KOLIN. (Introduced by L. N. KATZ.)

From the Cardiovascular Laboratory, Department of Physiology, Michael Reese Hospital, Chicago.

The Rein "Thermostromuhr",¹ the best method available for measuring flow in unopened vessels, is limited to mean flow measurements. In this laboratory a new method was developed which can follow rapid flow changes and which, besides, possesses the further advantage that the deflection bears a linear relation to the flow.

This flowmeter is based on the principle that an electromotive force is induced in a conductor moving so as to cut the lines of force in a magnetic field. If a wire is moving through the field in a direction perpendicular to its own axis and to the lines of force, then the

* This work was supported by a grant from the Rockefeller Foundation.

¹ H. Rein, *Z. f. Biol.*, 1928, **87**, 394.

potential difference at the ends of the wire is given by the expression:

$$E = H \cdot l \cdot v \cdot 10^{-8} \text{ volts}$$

where E denotes the induced voltage, H is the strength of the magnetic field, l , the length of the wire and v , its velocity.

A steady potential difference can be obtained by moving an endless metal belt, mercury or an electrolytic solution between the magnet poles. Here l would indicate the width of the conductor used. The induced electromotive force generated in the case of the solution can be tapped by introducing 2 non-polarizable electrodes through the wall of the tube through which the liquid is flowing. Since the induced voltage is proportional to the velocity of fluid flow, the current flowing through the galvanometer connected to these electrodes will be a linear function of the rate of flow. This was verified by actual trial with solutions of various concentrations of copper sulphate and sodium chloride using copper electrodes for the former and silver-silver chloride electrodes for the latter.

In the case of the blood vessels, their walls have a sufficient electrical conductivity to permit placing the electrodes outside in contact with their walls without opening them. This was demonstrated on

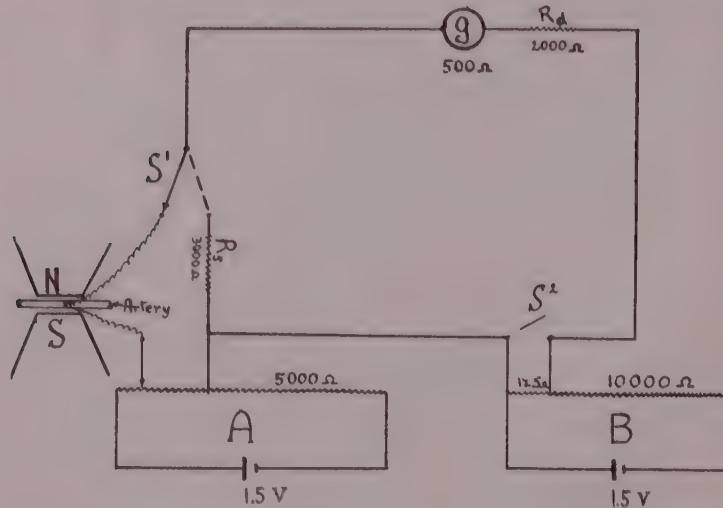


FIG. 1.

Scheme of the Flowmeter. N and S , magnet poles, one of the electrodes on the artery is seen between N and S , the other is hidden behind the artery; A , voltage source for compensating steady galvanic potentials; B voltage source for resistance measurements of artery with R_s , substitute resistance; S^1 , double throw switch; S^2 , shunt, G , galvanometer; R_d damping resistance.

arteries and veins, obtained postmortem, when perfusing them with saline.

The circuit used is shown diagrammatically in Fig. 1. The artery with the electrodes is shown between the two poles. The electrodes used consisted of zinc wrapped in cotton and dipped into a saturated solution of $ZnSO_4$. The electromagnet used had a magnetic field strength, when the poles were 4 mm. apart, of approximately 15,000 gauss, and the sensitivity of the D'arsonval galvanometer used was 1.3×10^{-8} Amp./cm.

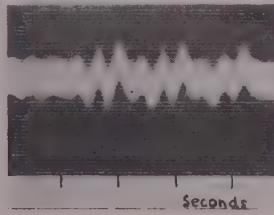


FIG. 2.

Fig. 2 shows the response of an Einthoven string galvanometer (sensitivity 0.1 millivolt = 1 cm.) to arbitrary pulsations of flow caused in the saline passing through an isolated carotid artery (reduced $\frac{1}{2}$). The method employed is free of any inherent inertia

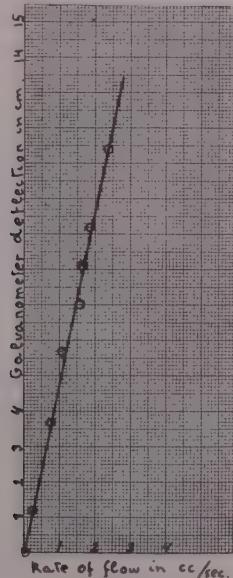


FIG. 3.

because it is based on a purely electro-magnetic effect. The limit of accuracy is determined by the characteristics of the galvanometer used.

Fig. 3 shows a typical calibration curve obtained with a carotid artery taken from a dog. The deflection difference observed on reversing the magnetic field was plotted against the rate of flow as measured with a graduate and stopwatch. It shows the linear relationship and gives an idea of the order of sensitivity of the particular arrangement.

The method was tried in living anesthetized dogs by placing the exposed but unopened carotid artery between the magnet poles. Deflections of as much as 14 cm. were obtained. The normal deflection was about 8 to 10 cm. With this sensitivity it is possible to determine changes in rate of flow of about 1% of the normal flow.*

I am indebted to K. Jochim for his valuable assistance and advice, to A. Meyer and S. Gaddas for technical assistance, and especially to Dr. L. N. Katz for his stimulating interest and guidance in developing the method.

8855 C

Effect of the Testis on the Mammary Gland.

C. S. McEUEN, H. SELYE AND J. B. COLLIP.

From the Department of Biochemistry, McGill University, Montreal, Canada.

We reported¹ that treatment with testosterone benzoate stimulates the development and secretion of the mammary gland in normal and castrate male and female rats. While following up these experiments, it became obvious that a certain degree of mammary gland development is always present even in untreated male rats after they have passed puberty. It seemed of interest, therefore, to establish whether the male gonad would physiologically exert a specific stimulating effect on the mammary gland. The experiments which we wish to report on here prove this to be the case.

A group of 12 immature castrate males, 34 days old, and 12

* These results indicate that electrical currents are induced in the body by the earth's magnetic field. Calculations suggest that the order of magnitude of the voltage induced in an aorta is of the order of 10^{-7} volts.

¹ Selye, H., McEuen, C. S., and Collip, J. B., PROC. SOC. EXP. BIOL. AND MED., 1936, **34**, 201.

normal controls of the same age were used in the first series. Biopsies of the mammary glands taken on the 48th day of life showed considerable glandular development and some milk secretion in the group of normals, while the castrate animals showed no secretion or development. A second biopsy, taken on the 56th day of life, showed that the development of the mammary gland in the non-castrate group had proceeded farther, while the castrates still showed no trace of development.

In order to see whether removal of the testes would cause involution of an already developed mammary gland, 6 adult male rats were castrated at the age of $4\frac{1}{2}$ months; that is, at a time when the mammary gland is already well developed. Biopsies 15 days later showed no secretion and marked signs of involution of the gland, which involution was still more advanced in biopsy specimens taken 30 days after operation.

In connection with this mammary gland stimulating effect, which is so similar to that of oestrin, it seemed of importance to establish whether testosterone would exert an oestrogenic effect on the vagina. This seemed all the more important since Deanesly and Parkes² state that testosterone causes cornification of the vagina, while Korenchevsky³ obtained vaginal mucusifications but no cornification.

Three normal and 2 ovariectomized 21-day-old females were injected subcutaneously once daily with 200 γ of testosterone in corn oil for 7 days. The vaginal smears showed no cornification. The

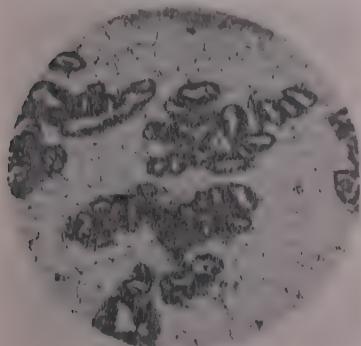


FIG. 1.

Mammary gland of a normal male rat,
48 days of age.

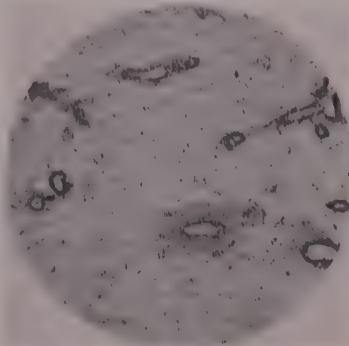


FIG. 2.

Mammary gland of a castrate male rat,
48 days of age.

² Deanesly, R., and Parkes, A. S., *Br. Med. J.*, 1930, 3018, 257.

³ Korenchevsky, V., *Nature*, 1936, 137, 494.

dose was then raised to 400 γ per day for a period of 4 days, and even this dosage had no vaginal oestrogenic effect, while the mammary glands exhibited marked development and secretion at the end of this period.

From these experiments it seems evident that testosterone is not oestrogenic in doses sufficient to stimulate the mammary gland. It is obvious, furthermore, that the effect of the male hormone on the mammary tissue cannot be attributed to its transformation in the organism into the female sex hormone. We have to conclude that testosterone itself stimulates the mammary gland both in the male and in the female, and that such mammary stimulation is a physiological result of the activity of the male gonad. In this connection clinical observations of Jung and Shaffron¹ are of interest. These authors found that proliferation of mammary tissue is normally demonstrable in boys between 12 and 17 years of age.

Summary. Development and secretion of mammary parenchyma is normally demonstrable in male rats beginning at puberty. Castration prevents the proliferation of the mammary gland in the immature male, just as it does in the female. In adult males, in which the mammary gland is already developed, castration is followed by involution of the gland. Testosterone in doses sufficient to produce mammary gland development does not cause vaginal cornification either in the normal or the ovariecomized immature female rat.*

¹ Jung, F. J., and Shaffron, A. J., *Proc. Soc. Expt. Biol. and Med.*, 1933, 282, 453.

* The testosterone borate used in these experiments was kindly supplied by the Schering-Kalbbaum Co., through the courtesy of Prof. Schoeller.

8856 P

Effect of Lactogenic Hormone Injections on the Crop Gland of the Hypophysectomized Pigeon.*

E. T. GOMEZ AND C. W. TURNER.

From the Department of Dairy Husbandry, Missouri Agricultural Experiment Station.

In a single case of a hypophysectomized ring dove, Riddle, Bates and Dykshorn¹ reported an increased weight of the crop gland to the extent of about 300%, with secretion of "crop-milk" following 4 daily injections of prolactin. Observations in our laboratory have shown that the injection of purified lactogenic hormone, galactin, immediately after hypophysectomy in cats, rats and guinea pigs, failed to initiate or prevent the rapid cessation of lactation. It is of interest, therefore, to determine whether galactin would cause the proliferation and the secretion of "crop-milk" of the crop glands of hypophysectomized pigeons. Common pigeons were used. The operation, which was carried on by the oral or parapharyngeal route, was well tolerated and the high post-operative mortality was minimized by the routine injection of glucose solution. In all instances, the injections of lactogenic hormone were started in 2 to 3 days after hypophysectomy, by the intramuscular or intradermal route for 4 consecutive days, the birds being sacrificed 24 hours after the last injection.

Of a total of 28 operated pigeons, 16 were found to be completely and 12 incompletely hypophysectomized at autopsy. The crop glands of the completely hypophysectomized birds which received galactin in amounts ranging from 4 to 10 mg. intramuscularly or intradermally over the crop sac all showed no evidence of proliferation. Three of the incompletely hypophysectomized birds which received 5 mg. and 2 which received 10 mg. of galactin intramuscularly, showed proliferation of both crop glands with an average rating of plus 2.6 and 1.25, respectively, while 7 which received 4 mg. intradermally over the crop sac showed proliferation only in the gland immediately above the site of injection. The response of these glands was rated as averaging plus 2 (2+). From these observations it was concluded that the purified lactogenic hormone, galactin, is incapable of causing the proliferation of the crop glands of hypophysectomized pigeons.

* Contribution from the Department of Dairy Husbandry, Missouri Agricultural Experiment Station, Journal Series No. 480.

¹ Riddle, O., Bates, R. W., and Dykshorn, S. W., *Am. J. Physiol.*, 1933, **105**, 191.

Galactin Content of the Rat Pituitary.*

R. P. REECE AND C. W. TURNER.

From the Department of Dairy Husbandry, Missouri Agricultural Experiment Station.

We reported¹ that the normal male rat pituitary contains an appreciable amount of the lactogenic hormone and that the injection of the estrogenic hormone definitely increases the galactin content of the pituitary gland.

Employing the same technique as that which we described in the paper referred to above we have extended the study of the galactin content of rat pituitary glands. The results are summarized in Table I.

From the table one can see that as the male rat continues to grow the pituitary gland likewise increases in weight. The bird units of galactin per pituitary gland increase as the animal matures, but this is due largely to the increase in the weight of the gland as one notices that the galactin concentration remains quite constant. The daily injection of 500 international units of Oestroform B into male rats for 7 days increases the weight of the pituitary gland and increases the galactin content of the gland while the content in bird units of galactin per mg. of fresh pituitary tissue remains nearly constant. The injection of thyroxine in increasing dosages decreases the weight of the pituitary glands, decreases the total potency of the glands, and decreases the galactin concentration per mg. of fresh pituitary tissue. The injection of thyroxine at the same level each day, 0.01 mg., produces the same results but to a less extent. Male rats castrated for 60 days possess much larger pituitary glands: the bird units of galactin per pituitary gland, however, remain constant.

The pituitary gland from the immature female rat contains about 3 times the amount of galactin of the immature male rat, this increased galactin content being accompanied by an increased glandular weight and an increased concentration of the galactin. As the female rat matures there is an increase in weight of the pituitary gland as well as an increase in concentration of galactin. At 12 days of pregnancy there is a decrease in the number of bird units

* Contribution from the Department of Dairy Husbandry, Missouri Agricultural Experiment Station, Journal Series No. 481.

¹ Reece, R. P., and Turner, C. W., PROC. SOC. EXP. BIOL. AND MED., 1936, 34, 402.

TABLE I.
Galactin Content of the Rat Pituitary.

| No. of Animals | Sex | Physiological Condition | Aver. body wt., beginning of expt., gm. | Aver. body wt. when sacrificed, gm. | Aver. pituitary wt., mg. | Bird units per pituitary gland | Bird units per 1 mg. pituitary tissue | Bird units per 100 gm. body wt. |
|----------------|-----|---|---|-------------------------------------|--------------------------|--------------------------------|---------------------------------------|---------------------------------|
| 10 | M | Normal | | 84 | 2.0 | 0.30 | .150 | 0.35 |
| 9 | M | 500 I.U. of Oestroform B/day for 7 days | 161 | 176 | 4.4 | 0.80 | .182 | 0.45 |
| 7 | M | Normal | 161 | 172 | 6.2 | 1.10 | .177 | 0.64 |
| 7 | M | .01 mg. thyroxine for 7 da., .02 mg. 7 da., .03 mg. 7 da. | 149 | 172 | 4.9 | 0.86 | .176 | 0.50 |
| 10 | M | Normal | 150 | 161 | 4.0 | 0.39 | .097 | 0.24 |
| 10 | M | .01 thyroxine/day for 14 da. | 319 | 318 | 8.1 | 1.28 | .158 | 0.40 |
| 10 | M | .01 thyroxine/day for 14 da. | 310 | 312 | 7.8 | 1.10 | .141 | 0.35 |
| 14 | M | Normal | 201 | 239 | 5.4 | 1.04 | .193 | 0.44 |
| 14 | M | Castrated for 60 days | 202 | 239 | 9.9 | 1.00 | .101 | 0.42 |
| 10 | F | Normal | 76 | 76 | 3.3 | 0.94 | .284 | 1.24 |
| 14 | F | , | 170 | 84 | 8.4 | 3.87 | .461 | 2.27 |
| 9 | F | 12 days pregnant | 180 | 81 | 8.1 | 2.90 | .358 | 1.61 |
| 8 | F | 21 , , , | 214 | 7.7 | 7.7 | 3.50 | .455 | 1.64 |
| 11 | F | 48 hrs. postpartum | 201 | 19.6 | 7.72 | .728 | .384 | 3.84 |
| 9 | F | 10 days | 206 | 9.7 | 5.86 | .600 | .284 | 2.84 |

of galactin per pituitary gland as compared with glands from normal estrous cycle females, and at 21 days pregnancy there is an increase in potency of the glands as compared with those from rats pregnant for 12 days. Forty-eight hours postpartum there is a doubling of the galactin content of the pituitary gland over that of the glands from either the normal estrous cycle female or the female pregnant for 21 days. At 10 days postpartum the galactin content of the glands decreases somewhat, being about midway between the glands from 48 postpartum rats and glands from normal estrous cycle females.

8858 P

Arbutin Diabetes.

FRANCES Y. MICHEL,* (Introduced by H. E. Himwich.)

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of the Albany Medical College.

The site of action of phlorhizin in phlorhizin diabetes is now recognized as being in the kidneys,¹ the disturbance caused by the drug being apparently due to inability of the tubule to reabsorb glucose. This, according to Lundsgaard,² arises from the fact that the poisoned tubule is unable to carry out phosphorylation of glucose. Moreover, several glucosides, including phlorhizin, are now known to inhibit phosphorylation *in vitro*, and glucose resorption in the isolated perfused frog kidney is also inhibited.³ It occurred to us that the glycosuric effect of phlorhizin might be a property common to all these glucosides. The action of arbutin, amygdalin, and salicin on healthy, fasting dogs was studied according to the procedure and methods of urine and blood analyses customary with phlorhizin.

Arbutin is, like phlorhizin, a phenol glucoside. When injected subcutaneously in sterile distilled water twice daily in fasting dogs, it caused a diabetes similar to that produced by phlorhizin with lowered blood sugar and acetone bodies in the urine, the ratio of

* These data are to be submitted to the Graduate School of Yale University in partial fulfillment of the requirement for the degree of Doctor of Philosophy.

¹ Deuel, H. J., Jr., Wilson, H. E. C., and Milhorat, A. T., *J. Biol. Chem.*, 1927, **74**, 265.

² Lundsgaard, E., *Biochem. Z.*, 1933, **264**, 221.

³ Abderhalden, E., und Effkeman, G., *Biochem. Z.*, 1934, **268**, 461.

dextrose to nitrogen in the urine being 1.45, 1.56, 1.50, and 1.92 in 4 different dogs. The urinary reducing substance was dextrose, for after yeast fermentation the urine no longer reduced Benedict's reagent. In contrast to the diuresis of phlorhizin diabetes, the urine of the animals receiving arbutin, likewise of those receiving the amygdalin and salicin, was very scanty unless water was administered by stomach tube, but the voluntary intake of water also was very small.

TABLE I.
Typical Protocol: Dog A1.

| Date 1935 | Arbutin gm. | cc. | Dextrose gm. | Nitrogen gm. | D:N | Acetone bodies mg./spec. | Blood Sugar mg. % |
|--------------|----------------|------|-------------------------|-----------------|----------|--------------------------------|-------------------------|
| 7/6 | 2.5+2.5 | 0 | | | | | |
| 7 | 5 | 0 | | | | | |
| 8 | 2.5+2.5 | 0 | | | | | |
| 9 | 2.5+5 | 310 | 4.53 | 12.88 | (.35) | — | |
| 10 | 5+5 | 490 | Urine discarded (stool) | | | — | |
| 11 | 5+5 | 210 | 4.47 | 3.38 | | | |
| | | | 4.00s | 3.29s | 1.22s | — | |
| 12 | 5 | 250 | 4.10s | 5.35s | .77s | 41.4 | 85 |
| 13 | 5+5 | 265 | 3.63s | 4.54s | .80s | 204.0 | 60 |
| 14 | 5 | 285a | 9.63s | 3.94s | 2.44s | 197.3 | |
| | | 120b | 2.32 | 1.89 | 1.23 | 56.7 | |
| 15 | 5+5 | 85 | 2.68s | 1.15s | 2.33s | 83.8 | 67 |
| 16 | 5+5 | 260 | 7.15 | 4.72 | 1.51 | 324.0 | |
| 17 | 0 | 280 | 5.86s | 4.57s | 1.28s | 266.0 | 55 |
| 18 | 0 | 90 | | | | | |
| 19 | 0 | 110 | | | | | 56 |
| | | | | | 11.48 | | |
| | | | | | Av. 1.45 | | |

Injections on 7/6 through 7/11 made with oil suspension of drug; all injections subsequent to this made with aqueous solution.

s indicates determinations made on filtrate after Somogyi precipitation to remove fecal proteins.

a—urine collected at 9:30 a.m. contaminated with feces.

b—urine collected at 1:00 p.m. clear.

Female German Shepherd, fasted 2 days before injection.

Three fasting dogs injected subcutaneously with salicin, an alcohol glucoside, in sterile distilled water exhibited no glycosuria nor ketonuria; nor did 3 dogs similarly injected with amygdalin, an aldehyde glucoside.

Lundsgaard⁴ found that phlorhizin inhibits glycolysis of excised tissues. Arbutin, like phlorhizin, appears definitely to inhibit glycolysis in brain, striated and cardiac musculature (0.1% glucose in double molar concentration Sorenson phosphate buffer of pH 7.4).

⁴ Lundsgaard, E., *Biochem. Z.*, 1933, **264**, 209.

TABLE II.
Effect of Arbutin (M 25) on Glycolysis Lactic Acid mg. %.

| Time min. | Brain | | Striated Muscle | | Cardiac Muscle | |
|--------------|---------|------|-----------------|------|----------------|------|
| | Without | With | Without | With | Without | With |
| 0 | 120 | | 54 | | 162 | |
| 30 | 422 | 160 | 257 | 101 | 342 | 250 |
| 90 | 430 | 246 | 373 | 143 | 372 | 146 |

In contrast with pilorhizin and arbutin, the results with salicin and amygdalin were inconclusive.

Since glycolysis may involve dehydrogenation at some point in the process, the effect of these drugs on the reduction time of methylene blue by rat kidney slices with and without glucose and sodium lactate was studied according to the Thunberg technique. All 4 drugs retard the reduction time with and without added substrate. The results are shown in Table III.

TABLE III.

| Kidney slices | Without added substrate | | With glucose | | With lactate | |
|------------------|-------------------------|-----------|--------------|-----------|--------------|-----------|
| | A | B min. | A | B min. | A | B min. |
| Without drug | 12 | 38 | 13 | 36 | 11 | 27 |
| M 100 Pilorhizin | 8 | 74 | 9 | 65 | 9 | 53 |
| M 25 Arbutin | 17 | 67 | 17 | 58 | 18 | 48 |
| M 25 Salicin | 12 | 75 | 13 | 58 | 12 | 45 |
| M 25 Amygdalin | 12 | 66 | 13 | 66 | 11 | 49 |

A—number of determinations.

B—average of the determinations listed in column A = average reduction time.

8859 P

Transformation of Bacterial Types.*

HOBART A. REIMANN.

From the Department of Medicine, Jefferson Medical College, Philadelphia.

In previous papers the isolation and description of numerous variant forms of *Micrococcus terragenus* was reported.¹ It was at first believed that these interchangeable variants composed a complex system of bacterial variation different from the orderly one associated with other bacteria. A clue to the solution of the prob-

* This work was done in the Department of Medicine at the University of Minnesota Hospital and was aided by a grant from the Graduate School Fund.

¹ Reimann, H. A., *J. Bact.*, 1936, **31**, 385, 407.

lem was provided many months later by the detection of typical rough colonies among the pink colonies already studied. An orderly arrangement of most of the variants and a basis for comparison with other bacteria was then made possible.²

With the rough-pink form at hand, this form and the mucoid-pink and pink were grouped together as the usual M, S, and R culture-phases of a given bacterial type. On this basis it was predicted² that the yellow, white, pink, pink-yellow, and brown forms represented distinct types of *M. tetragenus* and that each possessed the respective culture-phases. In subsequent investigation involving the aging of 100 cc. broth cultures of the types at hand for months at room-temperature, most of the predicted forms missing from the scheme were detected, isolated, and studied, namely, the mucoid-pink-yellow, mucoid-brown, rough-yellow, rough-pink-yellow, and rough-brown forms. The rough form of the white type has not yet been obtained although several methods known to favor dissociation were employed. The white type under these manipulations changed either into mucoid or translucent form and yellow colonies occasionally appeared. The studies will be published in detail elsewhere. The variant forms of *M. tetragenus* isolated were arranged as follows:

| | | | | |
|---------------|--------------|-------------|--------------------|--------------|
| Mucoid-yellow | Mucoid-white | Mucoid-pink | Mucoid-pink-yellow | Mucoid-brown |
| Smooth-yellow | Smooth-white | Smooth-pink | Smooth-pink-yellow | Smooth-brown |
| Rough-yellow | | Rough-pink | Rough-pink-yellow | Rough-brown |
| (G?) bacilli? | | | | |

It appears that *M. tetragenus* provides a unique opportunity for the study of type and culture-phase transformation. With most bacteria it would probably be difficult and laborious to determine type-transformation since colonies of different types are often indistinguishable morphologically; with *M. tetragenus* the types are marked by distinctive pigment which renders detection easy.

² Reimann, H. A., PROC. SOC. EXP. BIOL. AND MED., 1936, **34**, 344.

8860 C

Influence of Cattle Ant. Pituitary Extract on the Joints of Thyroidectomized Guinea Pigs.

MARTIN SILBERBERG.

From the Department of Pathology, Santo Tomas Hospital, and the National University, Panama, R. P.

In the joints of young guinea pigs changes corresponding to those seen in acromegalic arthropathy may occur under the influence of anterior pituitary extract of cattle. The lesions consist in a hypertrophy and hyperplasia of the cartilage cells, proceeding from the transitional zone towards the sliding or pressure zone and finally producing ulceration of the surface of the joint.¹ The newly formed cartilage may or may not undergo calcification. Inasmuch as thyroidectomy does not counteract the action of anterior pituitary on the epiphyseal line² and on bone repair,³ we wished to investigate what effects thyroidectomy would exert on the changes produced by anterior pituitary extract on the joints.

The investigations were made on 16 young guinea pigs which had already been used for study of the behavior of the epiphyseal line² and bone repair.³ Method and technique have been described previously. As a rule, the changes produced on knee and hip joints were studied by us.

As early as after 7 injections of the extract a proliferation of the cartilage cells is seen within the transitional zone of the thyroidectomized animals. The cells become hyperplastic, assuming a longitudinal arrangement in contradistinction to the flat horizontal position which they show normally. The cytoplasm of these cells hypertrophies, the nuclei of the cells are distinctly turgescent and mitotic figures are seen here and there. After 10 to 21 injections the cells have become still more numerous and the stroma between the cells is diminished. Their nuclei take on a more deeply bluish stain with hematoxylin as an indication of the relative immaturity of the growing cells. The distinct columnar arrangement of the cartilage cells is thus associated with these changes in the cells. The cell proliferation gradually extends towards the sliding zone of the cartilage and the surface of the joint. In addition, liquefaction of the cartilage takes place. Small ridges are formed, and in advanced

¹ Silberberg, M., PROC. SOC. EXP. BIOL. AND MED., 1936, **34**, 333.

² Silberberg, M., PROC. SOC. EXP. BIOL. AND MED., 1936, **33**, 554.

³ Silberberg, M., and Silberberg, R., PROC. SOC. EXP. BIOL. AND MED., 1936, **34**, 108.

cases we may even find gaps within the rapidly growing zone of cartilage. As soon as true ulceration has occurred, vascularization and ingrowth of bone-marrow into the cartilage cell layers is seen together with further destruction of the surface of the joint. These latter processes are especially frequent in the neighborhood of the insertion of the ligaments, where the cartilaginous covering of the joint is comparatively thin. However, these changes are also to be found more or less pronounced elsewhere on the surface of the joint. In addition, in some instances disturbances of calcification and of endochondral ossification are seen as revealed by a rapid calcification of the growing cartilage. Inflammatory changes are missing.

As to the arthropathic changes the following statement may be made: The greater the tendency to calcification of the newly-formed cartilage is, the more distinct is the ossification and the less is the ulceration which is to be expected. On the other hand, if the growth tendency of the cartilage predominates over calcification, the formation of ulcers will be more pronounced. Why in one case one effect is seen and the other effect in another case, is uncertain. However, the reaction does not depend upon the sex of the animals nor on the number of injections given. We see then that the changes observed in the cartilage of the joint under the influence of anterior pituitary extract in thyroidectomized guinea pigs take a course parallel to that seen in the epiphyseal line and in the chondrophyte under the corresponding conditions. Obviously, the cartilage of the epiphyseal line, of the chondrophyte and of the surface of the joint show the same growth reactions.

Conclusions. In thyroidectomized young guinea pigs anterior pituitary extract of cattle exerts its growth-promoting effect on the cartilage of the joint as well as in non-thyroidectomized animals, and the same arthropathic changes characteristic of acromegaly develop in both. Lack of the thyroid gland does not only not prevent the growth-promoting effect of anterior pituitary extracts, but in thyroidectomized guinea pigs the phenomena are even more pronounced than in normal animals which were treated with the extract. These experiments provide, therefore, further evidence for the conclusion that the extract exerts its influence on bone, epiphyseal line and joint cartilage without the intermediation of the thyroid gland.

Cardiac Glycogen in Diabetic Animals.

GERALD EVANS AND MORRIS A. BOWIE. (Introduced by C. N. H. Long.)

From the George S. Cox Medical Research Institute, University of Pennsylvania.

It has been shown previously¹ that cardiac glycogen is well maintained during fasting, exercise and epinephrine administration, in all of which liver and skeletal muscle glycogen is lowered. The present report gives the results of an examination of glycogen in the hearts of diabetic animals. In the literature there is evidence that cardiac glycogen is raised both in depancreatized animals^{2, 3, 4, 5} and in phloridzinized animals.^{6, 7, 8} However, in view of the wide range of values recorded and in view of the strict necessity for the avoidance of anoxemia in obtaining satisfactory values for cardiac glycogen,⁹ it was considered not out of place to repeat the observations, particularly since the point is of importance to further work.

Dapancreatized Cats. Three cats were depancreatized and maintained on insulin for from one to 2 weeks; food and insulin were then withdrawn, and after allowing 48 hours for the development of marked glycosuria, the animals were anesthetized with nembutal, artificial respiration established, and the chest and pericardium opened; the hearts were then secured by one rapid cut, immediately immersed in a roughly equivalent volume of cold 30% KOH and rapidly cut into approximately one-gram pieces. To obtain the weight of the sample, the vessel containing the KOH together with the scissors used in cutting the immersed heart was originally tared, and the whole reweighed after addition of the heart. After weighing, digestion was carried on for an hour on the steam bath and the analysis completed as previously described.¹ As controls, 3 hearts were similarly obtained from similarly fasted intact cats.

The values obtained for the 3 control hearts were 507, 410, 510 mg. 100 gm., average 476 mg. 100 gm.; those for the hearts of diabetic animals were 1345, 981, and 1496 mg. 100 gm., average

¹ Evans, G., *J. Physiol.*, 1934, **82**, 468.

² Cruickshank, E. W. H., *J. Physiol.*, 1913, **47**, 1.

³ Fisher, N. F., and Lackey, R. W., *Am. J. Physiol.*, 1925, **72**, 43.

⁴ Cruickshank, E. W. H., and Srivastava, D. L., *J. Physiol.*, 1930, **92**, 144.

⁵ Chambers, W. H., and Kennard, M. A., Pollack, H., and Dunn, M., *J. Biol. Chem.*, 1932, **97**, 525.

⁶ Junkersdorf, P., *Arch. f. d. ges. Physiol.*, 1923, **200**, 443.

⁷ Geiger, E., and Schmidt, E., *Arch. f. exp. Path. u. Pharmakol.*, 1928, **134**, 173.

⁸ Lawrence, R. D., and McCance, R. A., *Biochem. J.*, 1931, **25**, 570.

TABLE I
Effect of Phloridzin and Epinephrine on Cardiac Glycogen of Albino Rats.

| Experiment | No. of Animals | Glycogen, mg. 100 gm. | | | Blood glucose mg. 100 cc. |
|---|----------------|-----------------------|---------------|-------|---------------------------|
| | | Heart | Gastrocnemius | Liver | |
| <i>Controls—24 hr. fasted. No phloridzin</i> | | | | | |
| Phloridzin—24 hr. fasted | | | | | |
| " 48 " | 4 | 542 ± 19 | 361 | 188 | 84 |
| " 72 " | 4 | 481 | 323 | 66 | 66 |
| " 13 " | 13 | 538 | 367 | 73 | 57 |
| " 27 " | 4 | 498 ± 24 | 291 | 75 | 55 |
| " 33 " | 4 | 539 | 111 | 137 | 71 |
| <i>and 3 hr. after .02 mg. 100 gm. epinephrine subcutaneously</i> | | | | | |
| A preceding * except .04 mg. 100 gm. epinephrine | 4 | 528 | 105 | 83 | 64 |

1253 mg. 100 gm. The difference was so clear cut that it was not thought necessary to enlarge the series.

Phloridzinized Rats. Albino rats of 150-200 gm. weight were given subcutaneously 50 mg. of phloridzin daily in 1 cc. of olive oil. Food was removed at the time of the first injection. At the end of the fasting periods noted in the table the animals were anesthetized with nembutal and the gastrocnemius, heart and liver samples taken and analyzed for glycogen as previously described.² Glucose was determined in femoral vein blood by the colorimetric copper method of Benedict.³ To one group of 8 rats, after 24 hours of phloridzinization and fasting, epinephrine was given subcutaneously in the doses noted, and the animals sampled 3 hours later. The dilutions used were 1:50,000 and 1:25,000 and no delay was allowed to occur between the dilution of the stock Parke-Davis Adrenaline and its injection into the animals. The epinephrine was given because Geiger and Schmidt⁴ have reported that it raised cardiac glycogen in phloridzinized rats.

The table shows the results obtained and it appears from them that, although cardiac glycogen is well maintained during phloridzinization both with and without an accompanying injection of epinephrine, it is not raised above the control level.

In agreement with the findings recorded in the literature, cardiac glycogen is found to be much increased in depancreatized animals. In disagreement with other recorded findings, cardiac glycogen is found not to be raised as a result of phloridzinization either with or without injected epinephrine; a possible explanation is that an apparent increase may have been found as a result of the sluggish hearts of phloridzinized animals not suffering as great a loss of glycogen under brief anoxemic conditions as has been shown to occur with the more active hearts of normal animals.⁵

In view of the importance that has been assigned to insulin for glycogen deposition in the muscles of eviscerate preparations,^{10, 11} it is a surprising finding that cardiac glycogen is found to be well raised in the hearts of depancreatized animals, from which insulin is presumed to be absent; a long maintained high blood sugar level is the most obvious factor in favor of the increased deposition. On the other hand, low blood sugar, as in the case of the phloridzinized animals, did not result in a significant lowering of cardiac glyco-

² Benedict, S. R., *J. Biol. Chem.*, 1931, **92**, 141.

³ Best, C. H., Dale, H. H., Hoet, J. P., and Marks, H. P., *Proc. Roy. Soc. (London)*, 1926, **100B**, 55.

⁴ Choi, Y. O., *Am. J. Physiol.*, 1928, **83**, 406.

gen, although at the same time there was a marked decrease in the glycogen of skeletal muscle.

These two findings seem to indicate that heart glycogen is under different control than that of skeletal muscle: the alternate conclusion would be that if cardiac and skeletal muscle glycogen are under essentially the same control, then some additional factor not readily recognized when dealing with skeletal muscle is entering more largely in the case of the heart.

Cardiac glycogen is therefore set aside as an interesting subject for study, not only because of the possible importance to the heart itself, but because such an investigation might also bring new light to the views regarding carbohydrate and muscle metabolism generally.

Summary. Cardiac glycogen is found to be raised in depauperatized cats, and to be well maintained in fasting phenazinized rats, both with and without an accompanying injection of epinephrine. Attention is drawn to the importance of these findings to current views of carbohydrate change in the body.

8862 C

Effects of Chilling on Structure and Behavior of Embryos of *Amblystoma punctatum* Cope.

G. E. COGHILL.

Fallington, Pa.

In my study of the development of the behavior of *Amblystoma* it became imperative to know whether or not eggs or embryos that had been subjected to a low temperature, in order to prolong the experimental season by retarding growth, followed the normal order of development of movements. To meet this requirement I conducted experiments in 1930. Regarding behavior the results were conclusively in the negative, but it seemed desirable to vary the conditions in further experiments before publishing the results, especially in consideration of the numerous and extreme structural defects that appeared in the experimental animals. But inasmuch as I have not been able, and probably shall not be, to repeat the experiments the most obvious results are here presented on account of their bearing on experimental morphology. The work was done in the biological laboratory of the Effingham B. Morris Biological

Farm of the Wistar Institute of Anatomy and Biology. The special advantages of this laboratory are gratefully acknowledged.

Embryos from 2 clutches of eggs, designated 30M and 30E, were used for the experiments. They were collected from Gould's mill-pond in the Pocono Mts., Pa., April 13 and removed from the egg-masses, but not from the individual envelopes, on April 14. On April 20 they were placed in a mechanical refrigerator, the clutches in separate finger-bowls, where they remained till May 27. On this date the refrigerator was found not to be operating and defrosted, although it was operating on the previous day. The period of chilling was, therefore, 36 days. The temperature of the interior of the refrigerator fluctuated considerably with that of the room, which was also subject to rather marked changes according to the weather. However, the temperature of the embryos could not have been far above freezing for on one occasion (May 12) ice formed in the dishes.

30M. In this clutch there were 93 embryos between Harrison's stages 28 and 29 when placed in the refrigerator. April 29 2 had died. The others lived through the period of chilling. May 12 they had not reached stage 29. When finally removed from the refrigerator they were in approximately stage 34 and in apparently normal condition. They were then removed from the membranes. The ciliary action of the skin was strong but there was no muscular motility demonstrably in any of the specimens. Several specimens having been preserved for anatomical study, 71 were kept under observation. By June 3 structural abnormalities had appeared in 54; and by June 13, in all. The more obvious defects, and the number of embryos in which each occurred, were as follows: (A) gills more or less fused together, 41; (B) gills bent ventrad, 40; (C) tail bent dorsad near the middle, 35; (D) caudal fin reduced or wholly suppressed, 23; (E) trunk arched dorsad, 22; (F) abdomen transversely constricted, 20; (G) gills bent abruptly laterad at base, 15; (H) tail bent dorsad at tip, 13; (I) balancers bent caudad, 13; (J) tail bent dorsad at base, 10; (K) pericardium edematous, 7; (L) IV ventricle edematous, 6; (M) tail bent laterad, 4; (N) tail bent ventrad, 3; (O) arm fused with the body, 2; (P) gills bent abruptly dorsad, 1; (Q) balancer fused with the first gill, 1; (R) first gill completely suppressed, 1.

By June 13, 13 specimens had died. In these the distribution of defects was as follows: E in 10; D in 8; C in 6; I in 5; F in 3; A in 2; G in 2; B, M, N, P each in 1; H, K, L, O, Q, R, in none. Two to 5 of these defects occurred combined in single individuals.

30E. There were 45 embryos in this clutch. When put in the refrigerator they did not differ perceptibly from 30M in age; when removed they were in stage 31 or a little in advance of this. All survived the period of chilling in apparently normal condition. Forty-two were continued under observation for the effects of the treatment. The structural defects indicated above by letters occurred as follows: A in 31; B in 27; D in 16; C in 12; F in 10; I in 8; N in 7; H in 5; E in 4; R in 4; G in 3; O in 3; M in 1; Q in 1; J, K, L, and P in none. All had from 2 to 6 of these defects. In addition to these abnormalities, which appeared also in 30M, the balancers were grossly affected in 19, varying from short club-shaped structures to mere nodules. Nine died while under observation. In these the defects were distributed as follows: J in 8; D in 5; C in 4; E in 3; N in 2; A, B, G, and K each in 1; F, H, I, L, M, O, Q, and R in none. Beyond this period of special observation the surviving specimens were not given particular attention but it was noted that 14 of them were apparently normal individuals on the following Sept. 17. One of these had survived a combination of 6 of the above defects: A, C, D, I, M, N.

Obviously exact staging of these individuals according to Harrison was impossible. Nevertheless it was clearly established that in practically all the specimens the tail grew in length out of proportion to the dimensions of the head. This indicates that the ectodermal tissues were more retarded than the mesodermal. This difference in rate of growth of the 2 tissues offers an explanation of the structural defects of the gills, balancers, caudal fin, and fusion of the arm with the body wall. Also the dorsal curvature of the tail may have been due to longitudinal tension on the mesoderm due to a more slowly growing spinal cord. It is noteworthy that the embryos of 30E were more retarded during the period of chilling (probably due to a different position in the refrigerator) than those of 30M, and that the balancers were more affected in the former group. The edematous conditions were probably due to the failure of circulatory channels to open up in the normal manner because of some such retardation of growth as appeared in the transverse constriction of the abdomen. The death of approximately 20% of the specimens seems to indicate that in general the defects have more or less lethal action although the data are too limited for a statistical treatment of this question. However, a high degree of regulation is demonstrated.

The chilling in this case is recognized as relatively extreme in time and degree. Nevertheless the results demonstrate the need of exact studies of this nature with critical control of temperature if

chilled embryos of *Amblystoma* are to be used in experimental morphology, for retarding of the normal rate of growth by temperatures much below the optimum for the species must introduce variables of unknown value. At the same time this method may be used as a tool in experimental morphology in problems related to those attacked by Gilchrist,¹ and, correlated with cytological studies, it should yield significant results in regard to structure-function relations in development. It is known that localized acceleration of development within a relatively even gradient of growth in the central nervous system of *Amblystoma* accounts for the origin of its chief functional centers.² The development of the medullary plate also appears to take place according to the same principle within a wide field of potentially nervous ectoderm. The "organizer" in this case, and possibly in others, may be only an accelerator. The use of the method here proposed should throw new light on problems of this nature.

8863 C

Virucidal (Rabies and Poliomyelitis) Activity of Aqueous Urea Solutions.

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In an attempt to bring the suspended particles of an aqueous spinal cord suspension containing rabies or poliomyelitis virus into more intimate contact with inactivating agents for vaccine production trials a "solution" of the cord was made with the aid of urea. The effect of urea in strong concentration on these viruses proved interesting. As first recorded by Spiro¹ and in more detail by Ramsden² urea in aqueous solution has a remarkable ability to "dissolve" proteins. When spinal cord of the rabbit or monkey, moist with saline, is placed in a mortar and urea crystals added the cord on trituration quickly passes into an opaque syrupy "solution" containing innumerable lipid droplets in suspension. In this manner a 50%

¹ Gilchrist, Francis J., *J. Exp. Zool.*, 1933, **66**, 15.

² Coghill, G. E., *J. Comp. Neurol.*, 1926, **40**, 47; 1926, **42**, 1; 1928, **45**, 227; 1933, **57**, 327; 1936, **64**, 135.

¹ Spiro, Z. f. *Physiol. Chem.*, 1900, **30**, 182.

² Ramsden, W., *J. Physiol.*, 1902, **28**, xxiii.

cord "solution" containing from 30% to saturation with urea is easily obtained.

Rabies Virus. Typical results are presented in Table I. The fixed rabies virus usually killed within 6-11 days after intracerebral inoculation. The non-urea treated virus suspension was a 20% concentration of cord and brain in saline. The urea treated preparation contained 50% of cord-brain and about 40% of urea. Rabbits 3 and 4 were injected intracerebrally within an hour after the preparation of the cord "solution". They were given a second injection of 0.2 cc. each of a urea-treated preparation 10 days after the first ones with no ill effects. Rabbits 5 and 6 were given injections of 0.1 cc. each of the non-urea-treated preparation 3 weeks after the first injections. Both of them died within a week. Rabbit No. 7 was given a weekly "vaccination" by a 1.0 cc. subcutaneous injection of the 50% urea-treated rabies cord preparation for 6 weeks and then inoculated intracerebrally with the untreated suspension. Death followed shortly.

TABLE I.

| No. | Species | Weight Kg. | Treatment | Paralysis days | Death days |
|---------------------|-----------------------|---------------|---|-------------------|---------------|
| Rabies Virus. | | | | | |
| 1 | Rabbit | 2.1 | 0.1 cc. Rabies virus suspen. | 6 | 7 |
| 2 | " | 2.4 | 0.2 " " " " | 8 | 10 |
| 3 | " | 2.3 | 0.1 " Urea treated Rabies virus | 0 | 0 |
| 4 | " | 2.9 | 0.2 " " " " | 0 | 0 |
| 5 | " | 3.1 | 0.3 " " " " | 0 | 0 |
| 6 | " | 2.8 | 0.2 " " " " | 0 | 0 |
| 7 | " | 2.6 | 0.2 Rabies virus suspen. after "vaccination" | 7 | 0 |
| Poliomylitis Virus. | | | | | |
| Monkey | | | | | |
| Max. Temp. | | | | | |
| 8 | <i>Macacus rhesus</i> | 3.2 | 0.1 cc. untreated virus suspen. | 105.9 | 7 |
| 9 | " | 2.9 | 0.2 " " " " | 104.3 | 6 |
| 10 | " | 3.0 | 1.0 " urea treated virus | 101.7 | 0 |
| 11 | " | 4.1 | 0.5 " " " " | 100.5 | 0 |
| 12 | " | 2.6 | 0.5 " " " " | 102.1 | 0 |
| 13 | " | 3.3 | 1.0 " " " " | 100.8 | 0 |

Acute Anterior Poliomylitis Virus. Cords from monkeys dying after inoculation with M. V. (monkey passage) virus³ were used for preparing an untreated 10% suspension in buffered saline and a 40% "solution" in 40-50% aqueous urea. The results in Table I show that a strong urea solution inactivates poliomylitis virus. Two weeks after the injection of monkey No. 10 the animal was chloroformed and a brain suspension of the injected area injected

³ MacKay, E. M., and Schroeder, C. R., PROC. SOC. EXP. BIOL. AND MED., 1935, 83, 373

intracerebrally into another *Macacus rhesus* which did not develop the disease. Ten days after the first injection an accelerating dose^{4,5} of 1 cc. of the urea-treated preparation was injected into the brains of Nos. 12 and 13 and still no symptoms of poliomyelitis developed.

A female monkey weighing 4.8 kg. and a male monkey weighing 3.9 kg. were bled from the heart and their serums titered with a 5% poliomyelitis monkey cord suspension. In a 1-5 dilution the serum of both monkeys had antiviral substances to the extent that monkeys receiving the usual dose of the mixture by subdural injection were paralyzed but did not die. With a 1-10 dilution there was no protection whatever and both monkeys died. The 2 normal monkeys were then injected subcutaneously once a month for 3 months with 3 cc. of the urea-treated virus suspension. A month after the last injection, intracerebral inoculation with the regular virus suspension (0.2 cc.) resulted in their deaths in 7 and 10 days respectively. Serum from blood drawn just before these inoculations was titered just as before the "vaccination". At 1-5 dilution one monkey was paralyzed and the other died, while at 1-10 dilution both test monkeys died. We conclude from this that the strong solution of urea not only attenuates or dilutes the poliomyelitis virus in the sense that it is non-infective but actually destroys it, for it no longer possesses immunizing power.⁶

Urea is such a relatively inactive substance and certainly not a protoplasmic poison such as are most virucidal agents that it is in a way surprising that rabies and poliomyelitis viruses are killed so easily by urea solutions. Strong urea solutions have a certain degree of bactericidal activity⁷ but we have found that the death of bacteria usually requires considerably more time than that in which it kills viruses. The simple osmotic effect of the urea solutions can hardly be the cause of the virus death for glucose solutions of equivalent osmotic value will not inactivate rabies virus in any such period of time. It is true that neutral and inactive as it is, urea like alkalies denatures protein when dissolving it⁸ and this reaction may be associated with the death of the virus. This denaturation occurs in a very few minutes. It might be noted that the toxic globulin of *crotalus* venom or the vegetable protein ricin does not lose its toxicity when denatured by a strong concentration of urea.

⁴ Flexner, S., *Science*, 1931, **74**, 520.

⁵ Flexner, S., *Science*, 1933, **77**, 413.

⁶ Flexner, S., *Science*, 1935, **82**, 420.

⁷ Foulger, J. H., and Foshay, L., *J. Lab. and Clin. Med.*, 1935, **20**, 1113.

⁸ Hopkins, F. G., *Nature*, 1930, **126**, 328.

8864 P

Effects of Thorium on Blood and Liver Enzymes of White Rats.

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In a previous communication¹ some of the effects of thorium, zirconium, titanium and cerium on enzyme action *in vitro* were reported. Due to the peculiar physico-chemical properties of these metals, especially in respect to their precipitability in protein solutions it was deemed advisable to determine whether these effects could be observed *in vivo*. Roussy, Oberling and Guerin² have reported that thorium dioxide is carcinogenic when administered intraperitoneally to rats. In this investigation an attempt is being made to determine the effects produced by the intraperitoneal injection of varying amounts of soluble thorium nitrate, $\text{Th}(\text{NO}_3)_4$, on a variety of the blood and liver enzymes of the adult white rat.

The effects upon the following enzymes were investigated: blood amylase, blood lipase, blood esterase and blood phosphatase. The following liver enzymes were investigated: liver amylase, liver lipase, liver esterase, liver phosphatase, liver xanthine dehydrogenase, liver glucose dehydrogenase, and liver lactic dehydrogenase. Amylase was determined by the Willstatter-Schudel method,³ lipase and esterase by the method suggested by Sure *et al.*,⁴ phosphatase by the method described by Bodansky⁵ and the inorganic phosphorus determined by the method of Fiske and Subbarow.⁶ The dehydrogenase activities were estimated by the Thunberg technique as adapted by Collett.⁷

Twenty-one adult white rats were used for the study. Two sets of controls were run. One set of controls was inoculated with physiological saline and the other received an injection of dilute hydrochloric acid equivalent to the acid strength of the thorium injected into the experimental animals. All of the animals survived

* Contribution No. 80 from the Department of Biology and Public Health, Massachusetts Institute of Technology, Cambridge.

¹ Gould, B. S., *Proc. Soc. Exp. BIOL. AND MED.*, 1936, **34**, 381.

² Roussy, G., Oberling, C., and Guerin, M., *Bull. Acad. Med.*, 1934, **112**, 809.

³ Willstatter, R., *Unters. über Enz.*, Berlin, 1928, **1**, 2.

⁴ Sure, B., Kik, M. C., and Buchanan, K. S., *J. Biol. Chem.*, 1935, **108**, 27.

⁵ Bodansky, A., *J. Biol. Chem.*, 1933, **101**, 93.

⁶ Fiske, C. H., and Subbarow, Y., *J. Biol. Chem.*, 1925, **60**, 387.

⁷ Collett, M. E., *J. Biol. Chem.*, 1928, **78**, 685.

the injections of either thorium or acid although there was some sloughing at the point of inoculation when large doses of thorium or acid were introduced. The thorium-treated animals were divided into 2 groups, one receiving relatively small doses over a period of 7 days while the other received very large doses over a 5-day period. The total amount of thorium nitrate inoculated into the first group was 71.1 mg. and into the second group, 104.5 mg. The animals were killed by decapitation, the blood was collected and the serum removed for analysis. The liver was removed, washed, weighed and minced, then ground to a very homogeneous suspension. The animals were killed 1, 2, 4, 5, and 24 hours after the last inoculation.

From an analysis of the preliminary results obtained there seems to be no indication that the inoculation of thorium exerts any significant influence on the enzymic activity of the blood or liver of white rats. The results reported on the effects of thorium on phosphatase and amylase activity *in vitro* could not be observed *in vivo*.

The liver dehydrogenases show no detectable differences whether thorium or acid is introduced. Similarly, neither blood nor liver phosphatases show any variation on the thorium-treated or acid-treated animals.

Blood esterase is decreased by the injection of either thorium nitrate or acid. Similarly the liver esterase decreases after thorium or acid treatment. The variations in each case are comparable indicating that the differences are due not to the action of the metal but rather to the acid nature of the salt.

Blood and liver lipase were also slightly decreased on the injection of either thorium or the equivalent of acid. The effect was inappreciable one hour after inoculation and undetectable after 24 hours. After 3 hours there was an apparent but small decrease.

Liver amylase was unaffected by either thorium or acid. Blood amylase showed a small decrease in activity 2 hours after inoculation of either acid or thorium. After 4 hours there was still decreased activity but after 5 hours the amylase reached the normal level.

It appears that thorium nitrate in the quantities used exerts no effects upon certain blood and liver enzymes other than would be produced by the equivalent amount of acid.

8865 C

Emulsification of Fat for Intravenous Administration.

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An emulsion of fat that could safely be given in quantity by vein was first developed by Yamakawa and his collaborators¹ in Japan. Recent observations in Latin America and in this country² have confirmed and extended their work and have furnished very suggestive clinical and experimental evidence of the value of such preparations. The technique used by Japanese and American workers is essentially similar: the fat or oil is mixed with purified egg lecithin (Merck's or Kahlbaum's) and water and passed through a 2-stage dairy homogenizer operating at 3000 to 4000 lb. pressure, after which the product is sterilized by heat in sealed containers. When suitable quantities are used, it is possible to obtain an emulsion in which practically all the lecithin-coated fat globules are less than 2μ in diameter with only an occasional one as large as 3 or 3.5μ . Such an emulsion passes through the lung capillaries with ease,³ avoiding the danger of fat embolism, which may occur when the particles exceed 4μ in diameter.⁴

A difficulty that has been experienced—both with Japanese and American preparations—is the limited time during which these emulsions are stable. In the course of a few weeks, more or less, the larger fat particles which are in less active Brownian movement tend to rise to the surface, forming a visible cream layer. Although the formation of the cream does not necessarily indicate an increased particle size, nevertheless the large fat particles are in close apposition, and there is danger of their coalescing to form larger aggregates. The present studies were undertaken in an attempt to pro-

* Aided by a grant from Mead Johnson and Co., Evansville, Indiana.

¹ Yamakawa, *Nippon Naika Gakkai Zasshi*, 1920, **17**, 1, 22; Yamakawa and Nomura, *Jikken Iho*, 1928, p. 523; a series of papers in *Tohoku J. Exp. Med.*, 1928-1932, by Nomura, Baba, Sato, Hotta and others.

² Valledor, Casas, and Gomez del Rio, *Vida Nueva*, 1928, **23**, 156; Valledor, *Arch. de Med. des Enf.*, 1933, **36**, 276; Holt, Tidwell and Scott, *Am. J. Dis. Child.*, 1934, **48**, 926; *J. Pediatrics*, 1935, **6**, 151; Gordon and Levine, *Am. J. Dis. Child.*, 1935, **50**, 894.

³ Sato, *Tohoku J. Exp. Med.*, 1931, **18**, 120.

⁴ Markowitz and Mann, *Am. J. Physiol.*, 1931, **98**, 521.

duce emulsions which would be more stable, over a longer period of time, and hence more practical for intravenous administration.

It was found that a part of the difficulty was due to decomposition of lecithin under the influence of light and oxygen to form darker compounds; this change was also accompanied by the development of hemolytic properties. By using strictly fresh lecithin, sealing the material in nitrogen and keeping the preparations in the dark, some delay in creaming could be obtained.

The hydrogen ion concentration of the emulsion was next studied,[†] since it had been noted that the acidity increased on standing, and an attempt was made to prevent this by means of buffers. The introduction of a small amount of sodium bicarbonate sufficed to keep the pH above 6.0, and delayed the creaming but did not prevent it altogether. One might have feared that such addition of electrolyte would neutralize the charge on the fat particles and favor their aggregation, but such an effect was not observed at the concentrations employed. However, the addition of the sodium bicarbonate seemed to cause unfavorable reactions when the emulsion was injected into infants and it was therefore abandoned.

We then investigated the possibility that the interfacial tension between the lecithin and the fat might be a factor of significance in determining the stability of the emulsion. Measurements were made of the interfacial tensions between lecithin and olive oil mixtures, and we soon found, as had Okuneff,⁵ that a very small concentration of lecithin was sufficient to reduce the interfacial tension to an exceedingly low value. It was therefore felt that this was not a factor of significance in influencing the creaming. Our next step was to study the effect of varying the lecithin-fat ratio. The Japanese investigators had employed a ratio of 1:1.65, whereas Holt, Tidwell and Scott² had used a ratio of 1:2; we were unable to find any data as to the optimum ratio for purposes of emulsification. The virtue of lecithin as an emulsifying agent is supposed to lie in the fact that it contains both polar and non-polar groups, and theoretical considerations would lead one to believe that in such emulsions the lecithin forms a surface film bounding the fat globule. That the lecithin is indeed located at the surface of the particles was suggested by experiments in which emulsions were allowed to cream, the cream layer separated, and determinations of the lecithin-fat ratio made both in the cream layer containing larger particles and the underlying layer containing the smaller particles. In the latter,

[†] We wish to thank Dr. J. A. Pierce for these measurements, which were made with a quinhydrone electrode.

⁵ Okuneff, *Biochem. Z.*, 1928, **198**, 296.

a relatively larger surface would exist per unit volume of fat, and if the lecithin were at the surface, the lecithin-fat ratio should be higher in this portion of the emulsion than in the cream layer. This was actually found to be the case. Analysis of an emulsion which had stood for some time showed that the underlying unseparated layer had an L/F ratio of approximately 1:1 while the cream layer showed a ratio of approximately 1:2. Although the L/F ratio in the more stable part of this emulsion was of the order of 1:1, and although theoretical calculations of the ideal ratio based on rather crude estimates of average particle size pointed to this ratio as the ideal one, nevertheless we were not able to establish by experiment that such was the case. In general, stability seemed to improve with an increased proportion of lecithin, but no sharp limits were found. Even when the L/F ratio was carried beyond 1:1 the creaming difficulty was not obviated.

It seemed likely that the phenomenon of creaming could be obviated if the size of the particles could be sufficiently reduced, for as the size of the particles diminishes and the Brownian movement becomes more active, the influence of specific gravity is less effective in causing the particles to rise. With a homogenizer or a colloid mill it is not possible to reduce the size of particles beyond the dimensions described except by extreme dilution, which would have vitiated our purpose; we therefore turned our attention to another means of emulsification—namely the use of sonic and supersonic waves, first reported by Wood and Loomis.⁶ Our first attempts were made with audible sound of a frequency of 9,000 per second, generated by a magneto-striction apparatus[‡] as used by Chambers and Flosdorff.⁷

The materials to be emulsified were brought into contact with a vibrating nickel rod. Although considerable emulsification took place through the use of this method, it had to be abandoned because of the quantity of nickel that was suspended.

We then attempted to emulsify our materials by means of high frequency supersonic waves, generated from quartz crystals by means of the piezo-electric effect. Daniewski⁸ had demonstrated that the optimum frequency for emulsifying kerosene in water lay between 150,000 and 400,000 cycles per second. When Daniewski's curves are recalculated with power input as one of the variables it

⁶ Wood and Loomis, *Phil. Mag.*, 1929, **4**, 418.

[‡] This apparatus was kindly placed at our disposal by Drs. Leslie A. Chambers and Earl W. Flosdorff of the University of Pennsylvania.

⁷ Chambers and Flosdorff, *J. Biol. Chem.*, 1936, **114**, 75.

⁸ Daniewski, *Acta Physica Polonica*, 1933, **2**, 45.

is found that the rate of emulsification is proportional to power input. It therefore seemed desirable to use a frequency between these two figures with the maximum possible power input.

By means of such an apparatus, generating waves with a frequency of 300,000 cycles per second, it was found that fine emulsions of a high degree of stability could be obtained after rather prolonged radiation. § When an emulsion with a lecithin-olive oil ratio of 1:2 (total lipids 7%) was homogenized and sealed under nitrogen in 20 cc. thin-walled glass ampoules, very short periods of radiation did not appreciably retard the creaming. However when the ampoules were radiated for a total of 80-120 minutes, || a product was obtained which kept for 2 months and often more without the slightest evidence of creaming. The emulsion appeared slightly opalescent, rather than entirely opaque, and inspection under the darkfield microscope showed that practically all of the visible particles were 0.5μ in diameter, and none was larger than 1μ , even after 2 months.

An interesting observation was made in regard to the effect of the lecithin-olive oil ratio upon the result of the supersonic radiation. When a homogenized 6% water suspension of purified lecithin (containing very little egg oil) was used, 30 to 60 minutes of radiation produced a completely transparent solution in which no particles were visible in the darkfield. The gradual substitution of olive oil for part of the lecithin and the resulting gradual increase of the lecithin-olive oil ratio caused a progressive change from transparency to opalescence and then to the fairly opaque emulsion, observed at a 1:2 ratio, in which particles were visible in the darkfield.

We also studied the effect of omitting the preliminary homogenization in the dairy homogenizer before the supersonic treatment, and came to the conclusion that such preliminary treatment is necessary unless the proportion of lecithin is greatly increased. If the lecithin-fat ratio was greater than 2:1, a fine emulsion could be prepared by prolonged radiation of the crude (unhomogenized) mixture. However, when the standard 1:2 emulsion was radiated without homogenization, the supersonic waves failed to produce a stable emulsion. In fact, the occasional appearance of small oil droplets indicated that some of the coarse particles had been de-emulsified.

After preliminary injection of the radiated emulsion into rabbits

§ The preliminary experiments with this type of apparatus were conducted at the Loomis Laboratory, Tuxedo Park, New York, and were made possible through the courtesy and cooperation of Mr. Alfred L. Loomis.

|| This was done in 2-minute periods with cooling in between, to avoid possible heat effects.

had demonstrated that the supersonic preparation had produced no toxic substance, clinical observations were made in normal infants, the emulsion being given intravenously as described by Holt, Tidwell and Scott.² No untoward effects were observed. A study of the lipemic curve** after administration of one gram of lipid per kilo, indicated that the fat was removed from the blood in a normal manner. Comparisons of the rate of removal of the finer radiated emulsion with a somewhat courser unradiated emulsion showed that the radiated fine emulsion was not quite so readily rapidly removed at first. The difference, however, did not appear to be of any practical significance. Protocols of such a comparison are shown in Figure 1.

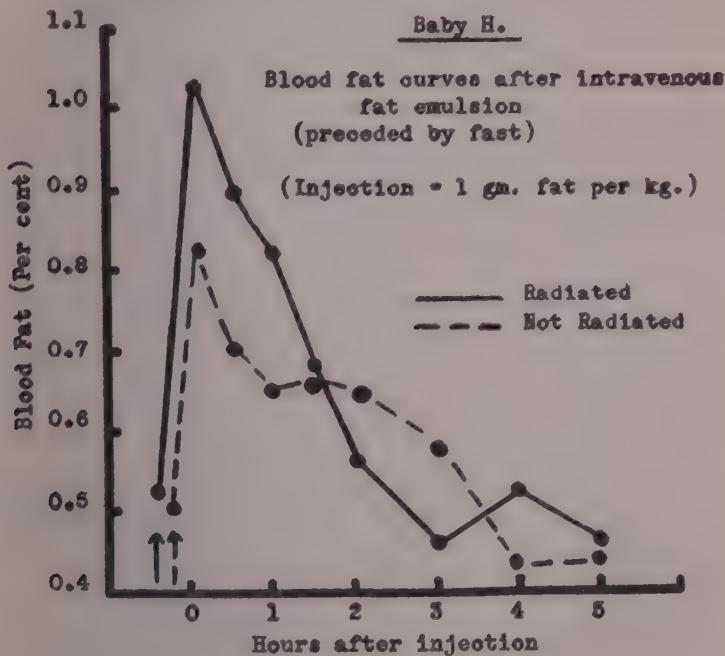


FIG. 1.

In addition to its efficiency in producing an emulsion of increased stability, the supersonic radiation method has a further advantage in that it permits autoclaving before the final step in the emulsification procedure. When emulsions are merely homogenized, they

** Blood fat determinations were made by the Gorter-Grendel micromethod (*Biochem. Z.*, 1928, **192**, 431).

must subsequently undergo a heat sterilization which usually causes some increase in particle size.

Conclusion. Supersonic radiation appears to be the most suitable method yet studied for preparing fat emulsions, suitable for intravenous use in man.

8866 C

Enlarged Tissue Cultures of European Typhus Rickettsiae for Vaccine Production.

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Our methods of producing typhus vaccines with the murine strains of Rickettsiae has depended upon the inoculation of rats in which resistance had been reduced by a variety of procedures, the most consistently successful of which has been preliminary X-ray radiation. These methods have persistently failed to give adequate results with the European virus. This fact in itself constitutes further strong evidence that the two types of Rickettsiae are distinct and biologically fixed varieties. After much effort to apply the "rat" methods to the organism of the classical European disease we were finally persuaded that other methods of approach must be sought for obtaining accumulations of the European Rickettsiae adequate for practical purposes of immunization.

In a paper, now in press, the writers have reported the results of experiments in which the active immunization of guinea pigs against the European typhus virus was accomplished, both with the use of formalinized tissue culture vaccines and by methods of sero-vaccination. While this work was going on, Kligler and Aschner¹ published observations on a method of successful animal immunization with formalinized tissue culture vaccine, similar in all important principles to the method which we are using.

Since the development of a tissue culture technique for producing vaccines in amounts adequate for practical purposes appeared the most hopeful direction of effort, we have tried for a long time to improve the technique of making such cultures, particularly in regard to increasing the volumes of the tissue cultures themselves.

*Guggenheim Fellow.

¹ Kligler, I. J., and Aschner, M., *Brit. J. Exp. Path.*, 1934, **15**, 337.

In studying the general problem of tissue cultures, one of the writers (Zinsser) in collaboration with Schoenbach, turned his attention to observations on what may be called the physiology of tissue cultures in general, particularly as regards changes in pH, oxygen consumption and oxydation-reduction potentials.† This work is far from complete and will not be reported until more extensive studies have been made. But as far as its preliminary results influenced our conception of Rickettsiae cultivation, it appears safe to make the following statements: In tissue cultures set up in the ordinary manner with guinea pig serum-tyrode solution and tunica vaginalis material from medium-sized guinea pigs, the oxygen consumption reaches its maximum and flattens out at the end of 40 to 46 hours. The potential distinctly and consistently rises, also reaching its maximum at about 40 hours. The pH usually shows a gradual change toward the acid side from an initial pH of about 7.8 to 7 to 7.2 at the end of 6 or 7 days. Thus, although the changes in the factors mentioned slow down and become more or less stabilized in such cultures before the end of the first 48 hours, the most active growth of viruses usually occurs after the 2nd day and that of Rickettsiae does not seem to take place until after the 5th to the 7th days. It would seem, therefore, that the conditions favoring growth depended upon the preliminary establishment of some sort of an equilibrium. It will not, of course, be possible to formulate any conception of what this equilibrium is or whether the measured changes have any direct significance, for the growth of Rickettsiae or virus agents, until far more extensive work along the lines mentioned has been carried out with cultures in which Rickettsiae and virus are actively growing. Even then physico-chemical interpretation may be impossible in systems so complicated.

Meanwhile, however, it seemed logical, in endeavoring to increase the volume and consequently the yield of typhus tissue cultures, to take these matters into consideration and to try to adjust culture volumes to air volumes in the larger flasks in a proportion comparable to that usually successful in the small 30 cc. flasks. In these, with 3 cc. of culture the cubic volume of fluid to the closed air space above it, is about 1 to 10. At the same time the amounts of tunica vaginalis tissue, while not actually measured were kept approximately proportionate to the larger amounts of fluid. In this

† Since in the ordinary smaller cultures the total oxygen consumption rarely exceeded 130 cubic millimeters, indicating a maximum of not over 800 in the larger cultures, it is most likely that the respiratory activity of the tissue is of less importance than the relative volumes of fluid and air placed in the sealed space.

way it is hoped that we might, by experimental trial and error, achieve an optimum at which the eventual ratio of oxygen and carbon dioxide in the culture fluid would favor growth. It may be stated that in earlier experiments, with large flasks in which we had paid no attention to these relations and had used only enough culture fluid to cover the bottoms in thin layers, either no growth or inadequate multiplication of Rickettsiae occurred.

The method as it is now successfully practiced is carried out in special flasks so constructed that the bottoms are flattened and the necks narrowed, the latter feature making it easier to seal and to avoid contamination. They are easily made from ordinary 250 cc. pyrex Erlenmeyers. Such a flask is represented in the figure. The volume of the altered 250 cc. flask is approximately 235 cc. not including the narrow necks.



FIG. 1.
Modified 250 Erlenmeyer flask for Typhus Tissue Culture.

Experiment has shown that the optimum amount of culture fluid for these flasks is about 20 cc. which gives a volume ratio of fluid to air space of 1 to 11 or 1 to 12 in different flasks. It is of the greatest importance to avoid an excess of tissue and to mince the tissue in such a manner that individual bits do not much exceed the size of a pin head. The tissue need not be accurately measured, but the proportion should be kept more or less as this is adjusted in the

ordinary Maitland cultures in the 30 cc. flasks as recommended by Nigg and Landsteiner.²

By using this method we are obtaining with reasonable regularity, considerable yields of European Rickettsiae in these large flasks. We have had positive results not only when the culture fluid consisted of guinea pig serum, one part to 3 or 4 of Tyrode's solution, but also when human serum or horse serum was mixed before filtration in proportions of 1 to 4 with the Tyrode solution. Indeed we seem to be getting our best Rickettsiae yields in guinea pig tissue with the horse serum Tyrode solution preparations. The best time for "harvesting" is on the 8th or 9th day of incubation at 37°C. As the cultures are going at present it should be possible to obtain 5 or more doses of vaccine from each of these flasks. And in view of observations that such tissue culture vaccines, treated with 0.1% formalin produce active immunization of guinea pigs against the European strain of typhus virus, the method offers practical possibilities for the mass production of such vaccines for trial on man.

8867 C

Serum Phosphatase in Cats with Total Bile Stasis.*

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The observation by Roberts of an increase in serum phosphatase activity in patients with jaundice has been confirmed by a number of investigators. Similar results have been obtained in dogs with experimentally produced obstructive and nonobstructive hepatic jaundice^{1, 2, 3} and elevated values have been reported in portal cirrhosis.^{4, 5} In view of the uniformity of the previously reported data, particularly in obstructive jaundice, and the importance of this

² Nigg, C., and Landsteiner, K., *J. Exp. Med.*, 1932, **55**, 563.

* Aided by the Jefferson Hospital Tumor Clinic and the Martin Research Fund.

¹ Bodansky, A., and Jaffe, H. L., *PROC. SOC. EXP. BIOL. AND MED.*, 1934, **31**, 1179.

² Hartman, F. W., and Schelling, V., *Arch. Path.*, 1934, **18**, 594.

³ Armstrong, A. R., and King, E. J., *Canad. Med. Assn. J.*, 1934, **31**, 14.

⁴ Greene, C. H., Shattuck, H. F., and Kaplowitz, L., *J. Clin. Invest.*, 1934, **13**, 1079.

⁵ Herbert, F. K., *Brit. J. Exp. Path.*, 1935, **16**, 365.

uniformity in connection with any attempt to explain the pathogenesis of this phenomenon and its significance, the results of our observations upon cats with total bile stasis are of interest.

Determinations of serum bilirubin concentration and serum phosphatase activity were made in 8 normal cats and 30 adult cats at various stages of total bile stasis produced by ligation of the com-

TABLE I.
Serum Bilirubin Concentration and Serum Phosphatase Activity in 8 Normal Cats
and 30 Cats at Different Periods of Total Bile Stasis.

| Cat | Stasis, days | Serum bilirubin, mg. per 100 cc. | Serum phosphatase units |
|-----|-----------------|-------------------------------------|----------------------------|
| 1-8 | 0 | 0.0 | 0.95-3.84 |
| 9 | 2 | 0.28 | 3.36 |
| | 4 | 0.41 | 2.39 |
| | 16 | 8.96 | 11.03 |
| 10 | 4 | 0.2 | 3.09 |
| 11 | 4 | 0.0 | 3.55 |
| 12 | 6 | 0.68 | 2.39 |
| | 13 | 4.8 | 3.76 |
| | 20 | 6.4 | 3.4 |
| | 29 | 2.52 | 5.92 |
| 13 | 6 | 3.64 | 3.21 |
| | 10 | 12.0 | 2.4 |
| | 21 | 24.0 | 5.8 |
| 14 | 7 | 2.32 | 3.72 |
| 15 | 7 | 0.0 | 3.8 |
| 16 | 8 | 2.04 | 4.6 |
| | 14 | 3.16 | 3.2 |
| 17 | 9 | 4.1 | 1.8 |
| 18 | 9 | 2.3 | 2.9 |
| 19 | 10 | 2.68 | 2.48 |
| 20 | 10 | 0.4 | 3.6 |
| 21 | 11 | 1.2 | 2.7 |
| 22 | 12 | 1.1 | 1.9 |
| 23 | 12 | 1.3 | 2.8 |
| | 20 | 8.2 | 3.4 |
| 24 | 13 | 20.2 | 1.07 |
| 25 | 14 | 5.2 | 1.25 |
| | 18 | 0.48 | 0.97 |
| 26 | 14 | 11.6 | 3.4 |
| | 21 | 19.2 | 2.7 |
| 27 | 15 | 1.61 | 3.1 |
| 28 | 15 | 0.98 | 3.78 |
| 29 | 16 | 0.32 | 3.82 |
| 30 | 16 | 0.41 | 1.84 |
| | 20 | 0.25 | 2.68 |
| 31 | 17 | 1.3 | 3.4 |
| | 19 | 1.18 | 2.93 |
| 32 | 17 | 0.64 | 2.04 |
| 33 | 18 | 0.32 | 1.96 |
| | 20 | 0.41 | 1.92 |
| 34 | 18 | 0.68 | 1.01 |
| 35 | 19 | 6.8 | 2.61 |
| 36 | 19 | 4.1 | 3.6 |
| 37 | 19 | 1.9 | 3.14 |
| | 21 | 1.8 | 3.81 |
| 38 | 20 | 0.8 | 2.24 |

mon bile duct. No case is included in which, at autopsy, there was evidence of the presence of any complication or any indication that stasis was not complete. The animals were maintained upon a diet of fresh raw scrap meat and milk. Serum bilirubin was determined by the method of van den Bergh and serum phosphatase by the method of Bodansky.⁶

The findings are presented in Table I. The control values ranged from 0.95 to 3.84 units of phosphatase. Of 45 determinations made upon 30 cats with total stasis, 41 during stasis of 2-21 days' duration were within the limits of the control values. Higher values were obtained in only 4 instances, as follows: 11.03, 5.92, 5.8 and 4.6 units, with stasis of 16, 29, 21 and 8 days' duration, respectively. There was no consistent relationship between serum phosphatase activity and either the duration of stasis or the degree of bilirubinemia.

These observations add another point of difference to those previously reported between the cat and other species in regard to the effects of total bile stasis upon the organism.⁷ The fact that a species difference does appear to exist in this respect indicates that care must be exercised in attempting to explain the mechanism of increase of serum phosphatase activity in obstructive jaundice in man and in dogs.

8868 P

Parathyroid Function in Hyperthyroidism.

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That abnormalities of function of other glands as well as of the thyroid gland occur in patients with hyperthyroidism is generally recognized. Conclusive evidence on the functional status of the parathyroid glands in hyperthyroidism, however, has not been reported. For this reason studies of parathyroid function by means of the Hamilton and Highman¹ test in patients with hyperthyroidism have been undertaken, and our findings to date are here presented.

⁶ Bodansky, A., *J. Biol. Chem.*, 1933, **101**, 93.

⁷ Cantarow, A., and Stewart, H. L., *Am. J. Path.*, 1935, **11**, 561.

¹ Hamilton, B., and Highman, W. J., Jr., *J. Clin. Invest.*, 1936, **15**, 99.

The Hamilton and Highman¹ test for parathyroid function was performed in 6 patients with hyperthyroidism, and in 5 control subjects (Table I). In this test,¹ 30 cc. of blood from a patient are injected intramuscularly in a rabbit from which a blood sample has been drawn for measurement of the concentration of calcium in the serum. At regular intervals thereafter calcium chloride solution is administered to the rabbit by stomach tube and blood samples taken for measurement of calcium. Interpretation of the test is based on the magnitude of the increase in the calcium of the rabbit's serum. Studies of the basal metabolic rate and of the concentration of calcium, inorganic phosphorus, and protein of the serum and of the plasma phosphatase activity were made in the hyperthyroid cases. The bony calcification of the hands of each patient with hyperthyroidism was compared with that of a normal subject of approximately the same age by taking a roentgenogram of both simultaneously. Blood for injection in the rabbit and for chemical studies was drawn from all subjects after an over-night fast. Basal metabolic rate measurements and phosphorus, protein and calcium measurements were performed and calculated according to methods referred to in other papers from this laboratory.^{2, 3} The method of Kay⁴ was utilized to measure plasma phosphatase activity. In one patient with hyperthyroidism a course of 2 weeks of iodine therapy was terminated 4 days before study; tests were performed both before and 5 days after the institution of iodine therapy in another case.

The basal metabolic rates in the patients with hyperthyroidism varied from +5% (iodine therapy) to +55% of normal; these patients were not markedly toxic with the exception of case 6 (Table I). The concentration of calcium in the serum was between 9.7 and 10.9 mg. % in 4 of the hyperthyroid cases; in one instance (Case 6), a thyrocardiac subject, it was 8.1 mg. %. The concentrations of protein and of inorganic phosphate in the serum were normal in each patient; the plasma phosphatase varied from normal to approximately one and one-half times normal (Table I).

The maximum increase in the calcium of the rabbits' sera after injection of blood from the hyperthyroid patients was above 0.2 mM. in 6 of the 7 studies (Table I), whereas in only one of 38 studies in normal subjects made by Hamilton and Highman¹ and in none

¹ Gilligan, D. R., Volk, M. C., Davis, D., and Blumgart, H. L., *Arch. Int. Med.*, 1934, **54**, 746.

² Gilligan, D. R., Volk, M. C., and Altschule, M. D., *J. Biol. Chem.*, 1933, **103**, 745.

⁴ Kay, H. D., *J. Biol. Chem.*, 1930, **89**, 235.

TABLE I.
Results of Hamilton and Highman Test for Parathyroid Hormone in Blood.

| Case No. | Age, yrs. | Basal Metabolic Rate, % deviation from standard normal | Plasma Phosphatase Activity, units | Serum Calcium of Rabbit, Maximum Increase, mM. per liter | Remarks |
|---------------------------------------|-----------|--|---------------------------------------|--|---|
| <i>Patients with Hyperthyroidism.</i> | | | | | |
| 1 | 12 | +34 | .34 | .23 | |
| 2 | 23 | +24 | .24 | .00* | |
| 3 | 30 | +34 | | .30 | |
| 4 | 24 | +31 | .11 | .32 | |
| 4 | 24 | + 5 | | .70 | Patient receiving Lugol's solution for 5 days before test. |
| 5 | 19 | +17 | .28 | .45 | Patient had been receiving Lugol's solution 2 weeks; omitted this 4 days before test. |
| 6 | 57 | +55 | .23 | .48 | |
| <i>Control Subjects.</i> | | | | | |
| 7 | 42 | +12 | .08 | .18 | Osteoporosis; etiology not known. |
| 8 | 35 | | | .00* | Normal subject. |
| 9 | 27 | | | .19 | " " |
| 10 | 25 | | | .08 | " " |
| 11 | 24 | | | .08 | " " |

*The concentration of calcium in the rabbit's serum both at the 3-hour and 5-hour sampling was less than that of the control specimen.

of the 5 control studies which we have made (Table I) was there an increase above 0.20 mM. The above results indicate abnormally large amounts of parathyroid hormone in the blood of 5 of the 6 patients with hyperthyroidism; the excess amount of parathyroid hormone can be quantitated only roughly in this small series of studies.⁵

That there are occasionally patients with hyperthyroidism who show marked generalized osteoporosis and that there exists a rather general tendency to increased calcium excretion in hyperthyroidism is well known.^{6, 7} The mechanism through which these abnormalities in calcium metabolism occur has not been clarified;^{7, 8} studies are being continued to evaluate the significance of increased parathyroid function as indicated by the findings here reported. There

⁵ Hamilton, B., Dasef, L., Highman, W. J., Jr., and Schwartz, C., *J. Clin. Invest.*, 1936, **15**, 323.

⁶ Aub, J. C., Bauer, W., Heath, C., and Ropes, M., *J. Clin. Invest.*, 1929, **7**, 97.

⁷ Hansman, F. S., and Wilson, F. H., *Med. J. Australia*, 1934, **1**, 37.

⁸ Albright, F., Bauer, W., and Aub, J. C., *J. Clin. Invest.*, 1931, **10**, 187.

was no definite X-ray evidence of osteoporosis in any of the cases of hyperthyroidism in this study; the phosphatase results gave evidence of only slightly increased osteoblastic activity in some instances (Table I).

Summary. Studies with the Hamilton and Highman test¹ of parathyroid function have been performed in 6 patients with hyperthyroidism. The results compared with those obtained in normal subjects indicate increased circulating parathyroid hormone in the blood of 5 of the 6 thyrotoxic patients. This investigation is being continued to evaluate the significance of the findings.

8869 P

Liver as a Possible Site of the Emetic Action of Strophanthidin in Cats.

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Hanzlik and Wood¹ published an important paper on digitalis emesis in which they contend, with Hatcher and others, that the mechanism of the emesis is a reflex one. They conclude that the liver is probably the chief site of origin of the reaction but that other abdominal organs may be involved, at least experimentally, because in pigeons with the liver excluded from the circulation digitalis still produces vomiting. They agree with Dresbach and Waddell² that the heart has been pretty definitely excluded as the chief site of origin of this response.

In this laboratory the possible relation of the liver to strophanthidin emesis in the cat has been attacked by two methods. In one the attempt was made to denervate the liver as thoroughly as possible and in the other the organ was completely removed. Complete denervation of the liver by surgical means may prove to be an impossibility (a study of this problem is being made) but at least it can be deprived of the greater part of its nerve supply. We have used no new technic but have stripped the duodeno-hepatic artery, portal vein, and common bile duct of all accompanying nerve fibers, which were cut, together with all other structures entering the liver

¹ Hanzlik, P. J., and Wood, D. A., *J. Pharm. and Exp. Therap.*, 1929, **37**, 67.

² Dresbach, M., and Waddell, K. C., *J. Pharm. and Exp. Therap.*, 1926, **27**, 9; *ibid.*, 1928, **31**, 43.

along the portal vein, and have painted the duct and vein with 70% alcohol, or with 2% phenol or silver nitrate; in 2 cats we also cut the right phrenic nerve just above the diaphragm. In spite of all of these procedures some undegenerated nerve fibers were found between the liver lobules, sufficient time having been allowed for degeneration. However, we do not know whether they were mainly afferent or efferent. Twenty-one animals have been operated, some of them very thoroughly; but the limitations of the technic have been appreciated.

With few exceptions the cats were in good condition when injected, by the saphenous and superior mesenteric veins, intramuscularly, intraperitoneally, or into a lobe of the liver. Except for novocain used locally in the intravenous injections, no other anesthetic was employed. Control injections of water, normal saline, and water containing a trace of alcohol were made in amounts comparable to those of the strophanthidin solution and by the same channels.

Pure crystalline strophanthidin, kindly supplied by Dr. Walter A. Jacobs, of the Rockefeller Institute, was used in the form of a water solution.*

Result. Vomiting was produced in every cat with the exception of one, which showed very definite nausea but it never occurred in the control experiments.

In a second series of 4 cats the liver was completely removed by the 2-stage method of Soskin.³ The method is comparatively simple and served our purpose well. No special attempt was made to prolong the lives of the animals after the final operation, although some of them were given glucose by vein. They survived from 5 to 12 hours.

One of these cats was injected 4 hr., 45 min., and one 9 hr., 13 min., after the excision of the liver. They received a total of 0.40 and 0.45 mg. per kg., respectively, in 2 doses about one-half hour apart, by muscle and the result was that both vomited repeatedly and in a perfectly typical manner after a latency of a little less than 40 minutes, this latency being well within the normal. One other cat was given 0.25 mg. per kg. by vein 3 hours

* The solution was made as follows: 25 or 50 mg. were dissolved in 3 or 4 cc. of 95% alcohol in a 50 cc. volumetric flask; this was filled about half-full with distilled water and placed in a water bath at 60° C. for 2 or 3 hours to drive off all but a trace of the alcohol; the volume was then made up to 50 cc. with sterile water and kept sterile. As one cc. of the solution contained 0.5 or 1.0 mg. of strophanthidin, only a very small amount of water was injected at any time.

³ Soskin, S., *J. Lab. and Clin. Med.*, 1931, **16**, 382.

after removal of the liver. Fourteen minutes later there was profuse salivation and after a latency of 47 minutes it almost vomited; at the end of 2 hours it died in a vomiting-like convulsion. The fourth cat, in apparently good condition, received 0.25 mg. per kg. by muscle 7 hr., 45 min., after liver removal. Although the dose was repeated twice a half-hour or so after the first injection no emesis resulted; however, there was marked tachypnoea.

From the results of the denervation work it is probable that if actual complete denervation of the liver in the cat could be attained strophanthidin would still cause emesis; the dehepatization experiments lend support to the conclusion. Although each of the 2 dehepatized cats which vomited received double the normal minimal emetic dose, in order to increase the probability of response, it does not necessarily follow that the operated cats were greatly depressed. Nevertheless, the negative results in the fourth cat suggest that the abnormal metabolic state had lowered the reactivity, as one would expect.

Conclusion. Strophanthidin can induce emesis in cats after very extensive denervation of the liver, and also after dehepatization, thus confirming the results Hanzlik and Wood obtained with pigeons. How the vomiting is brought about in animals deprived of the liver, or its nerve supply, is a problem for further study.

8870 C

Presence of Cholesterol in Combined Form in Human Bile.*

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Early reports of the presence of cholesterol esters in bile have been questioned because the methods used for the determination of esters were open to criticism. Later work by Thannhauser¹ indicates that human bile contains only free cholesterol. A recent paper by Wright² reported that the cholesterol present in dog bile is also present as free cholesterol. He was unable, using an improved method of analysis, to find any evidence of cholesterol esters. In

* Aided by a grant from the Josiah Macy, Jr., Fund.

¹ Thannhauser, S., *D. Arch. Klin. Med.*, 1922-1923, **141**, 290.

² Wright, A., *J. Exp. Med.*, 1934, **59**, 407.

the last 2 years, using a method which has been demonstrated to be satisfactory for determination of esters in bile,³ we have investigated a large number of bile specimens from patients with biliary fistulae. The data reported in this paper indicate that under rare conditions cholesterol may occur in human liver bile in a combined form.

With one exception all the patients from whom bile was collected had either had a cholecystostomy or choledochostomy. These patients had either simple gallstone disease, with or without cystic duct block, common duct stone, with or without obstruction, or malignant disease of the head of the pancreas. In one instance the biliary symptoms were very mild, but exploration was advised in the hope that the cardiac symptoms from which the patient suffered were in part due to chronic gallbladder disease. The gallbladder did not appear to be diseased when examined at operation, but a cholecystostomy was done following exploration of the interior of the gall bladder wall.

A bile sample was collected almost daily from each patient, and analyzed for concentration of free and total cholesterol. The method of analysis was that described by Riegel and Rose.³

In Table I are given the results of analyses of bile from a number of these patients. In all, the bile from 32 patients was studied, although not all analyses are included in the table.

It will be observed that of the 10 patients listed first in the table, none showed the presence of esters of cholesterol after the first few days. In each of these the bile for from one to 4 days after operation was contaminated with blood and we believe that the small amount of ester which was frequently found during this period can be attributed to the presence of blood in the bile specimens.

However, the patient, F. B., whose bile is that reported last in the table, presents a different picture. Here, cholesterol in the combined form was present in all the specimens collected up to the time the drainage tube was removed 17 days after operation. No contamination of this bile with blood was noted at any time, except possibly the very first specimen obtained immediately after operation.

Bile removed from this patient's gall bladder at operation also presented an unusual picture. The total concentration of cholesterol was 2480 mg. %.

The case described presents an unusual finding and one which we believe has not been reported previously in the literature. The patient, who differed from the others in that she had angina pec-

³ Riegel, C., and Rose, H. J., *J. Biol. Chem.*, 1936, **113**, 117.

COMBINED CHOLESTEROL IN HUMAN BILE

TABLE I.

| Patient | Bile obtained from | Days After | Cholesterol | | |
|---------|--------------------|---------------|----------------|---------------|----------------|
| | | | Total mg. % | Free mg. % | Ester mg. % |
| K.K. | Common Duct | 10 | 128 | 127 | 0 |
| | " " | 15 | 109 | 109 | 0 |
| | " " | 17 | 107 | 107 | 0 |
| | " " | 21 | 102 | 102 | 0 |
| | " " | 31 | 179 | 181 | 0 |
| | " " | 35 | 130 | 130 | 0 |
| G.S. | " " | 3 | 31 | 29 | 0 |
| | " " | 8 | 79 | 78 | 0 |
| | " " | 16 | 81 | 81 | 0 |
| A.S. | Gall Bladder | 2 | 50 | 50 | 0 |
| | " " | 10 | 202 | 200 | 0 |
| | " " | 15 | 188 | 186 | 0 |
| E.G. | " " | 13 | 112 | 110 | 0 |
| | " " | 17 | 175 | 174 | 0 |
| | " " | 19 | 136 | 135 | 0 |
| E.P. | Common Duct | 1 | 126 | 125 | 0 |
| | " " | 4 | 58 | 57 | 0 |
| | " " | 12 | 114 | 115 | 0 |
| | " " | 17 | 96 | 96 | 0 |
| | " " | 21 | 83 | 84 | 0 |
| W.W. | Gall Bladder | 4 | 38 | 36 | 0 |
| | " " | 6 | 35 | 34 | 0 |
| | " " | 10 | 26 | 26 | 0 |
| | " " | 12 | 20 | 21 | 0 |
| | " " | 15 | 11 | 12 | 0 |
| | " " | 18 | 29 | 28 | 0 |
| | " " | 21 | 23 | 23 | 0 |
| | " " | 28 | 50 | 50 | 0 |
| M.Y. | " " | 29 | 35 | 35 | 0 |
| | " " | 6 | 124 | 126 | 0 |
| | " " | 9 | 216 | 216 | 0 |
| | " " | 12 | 216 | 220 | 0 |
| D.B. | Common Duct | 5 | 43 | 44 | 0 |
| | " " | 8 | 133 | 134 | 0 |
| | " " | 9 | 150 | 150 | 0 |
| | " " | 29 | 198 | 197 | 0 |
| I.M. | " " | 2 | 26 | 26 | 0 |
| | " " | 18 | 53 | 53 | 0 |
| R.W. | " " | 14 | 9 | 8 | 0 |
| | " " | 18 | 12 | 11 | 0 |
| | " " | 31 | 15 | 14 | 0 |
| | " " | 36 | 14 | 15 | 0 |
| F.B. | Gall Bladder | 6 | 153 | 124 | +29 |
| | " " | 9 | 144 | 128 | +16 |
| | " " | 10 | 123 | 95 | +28 |
| | " " | 11 | 137 | 80 | +57 |
| | " " | 12 | 21 | 13 | + 8 |
| | " " | 13 | 126 | 82 | +44 |
| | " " | 15 | 98 | 82 | +66 |
| | " " | 17 | 62 | 27 | +35 |

toris, had only mild biliary symptoms, had no stones, no obstruction of the biliary tract, and had an apparently normal gall bladder and liver. The fact that bile from such a patient contained cholesterol in a combined form may indicate:

1. Patients with angina pectoris may have such an excess of cholesterol ester in the blood that the liver is unable to handle it, and hence some ester spills over into the bile. Total blood cholesterol of this patient, however, varied from 268 to 316 mg. % . While distinctly above the normal, the blood cholesterol cannot be considered excessively high. Of the 268 mg. per 100 cc. of blood, 86 mg. % was free, and 182 mg. % combined cholesterol (67% of total), which is considered a normal distribution.

2. It is possible that normal human bile may contain esters. The remainder of the bile specimens were from patients with diseased biliary tracts. Against this, is the fact that normal dog bile contains no ester. One might also expect that, as the liver began to recover from the effects of obstruction, cholesterol esters might begin to appear in the bile if they are normally present. This has never been observed in any of the patients we have studied, although specimens obtained as late as 191 days after operation have been analyzed.

3. Absence of the enzyme "cholesterolesterase" described by Thannhauser. This seems with our present knowledge the most logical deduction. Further studies of incubation of this bile with blood have a bearing on this phase of the subject and will be reported later.

Summary. In the majority of specimens of drainage bile obtained from biliary fistulae in the human patient, cholesterol is present only in the free form unless the bile is contaminated with blood. One case is reported of the occurrence of combined cholesterol in bile draining from a cholecystostomy.

8871 C

Acute Toxicity of Certain Sugar Alcohols and Their Anhydrides.*

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The narcotic effect of the sugar alcohols as a series was studied by Macht.¹ Recently the authors^{2, 3, 4, 5} have studied the fate of these substances and their anhydrides in the animal body and in bacterial culture media. In these communications, the chemical identity of the materials is established. The differences observed between the metabolism of the alcohols and their respective anhydrides prompted a study of their acute toxicities.

White mice (15 to 25 gm.) were employed. The compounds were prepared in solutions of the concentrations shown in Table I, and a total volume of not more than one cc. was injected intraperitoneally. That dose which killed one-half of the animals within a

TABLE I.

| Substance | Toxic Dose gm./100 gm. | % Solution | Mol Fraction $\times 10^{-4}$ | Character of Action |
|--------------------|---------------------------|---------------|----------------------------------|------------------------|
| Methyl Alcohol | 1.2-1.4 | 40 | 406 | Depression |
| Ethylene Glycol | 1.7-1.9 | 40 | 291 | " |
| Glycerin | 0.7-0.9 | 40 | 87 | Convulsion |
| Erythritol | 0.7-0.9 | 40 | 65 | " |
| Adonitol | 1.0-1.2 | 40 | 72 | " |
| Mannitol | 1.4-1.6 | 30 | 83 | " |
| Sorbitol | 1.5-1.7 | 40 | 87 | " |
| Glucose (control) | < 1.8 | 40 | — | " |
| Sucrose ("") | > 2.0 | 40 | — | " |
| Ethylene Oxide | 0.01-0.05 | 1 | 7 | Depression |
| Epiphydrin Alcohol | 0.05-0.10 | 5 | 10 | " |
| Erythritan | 1.7-1.9 | 40 | 173 | Convulsion |
| Mannitan | 1.6-1.8 | 40 | 104 | " |
| Isomannide | 1.5-1.7 | 40 | 109 | " |

* The expense of this investigation was defrayed in part by a grant from the committee on Therapeutic Research of the Council on Pharmacy and Chemistry of the American Medical Association.

¹ Macht, D. L., and Gin Ching Ting, *Am. J. Physiol.*, 1922, **60**, 496.

² Carr, C. J., Musser, R., Schmidt, J., and Krantz, J. C., Jr., *J. Biol. Chem.*, 1933, **102**, 721.

³ Krantz, J. C., Jr., Evans, W. E., and Carr, C. J., *Quart. J. Pharm. and Pharmacol.*, 1935, **8**, 213.

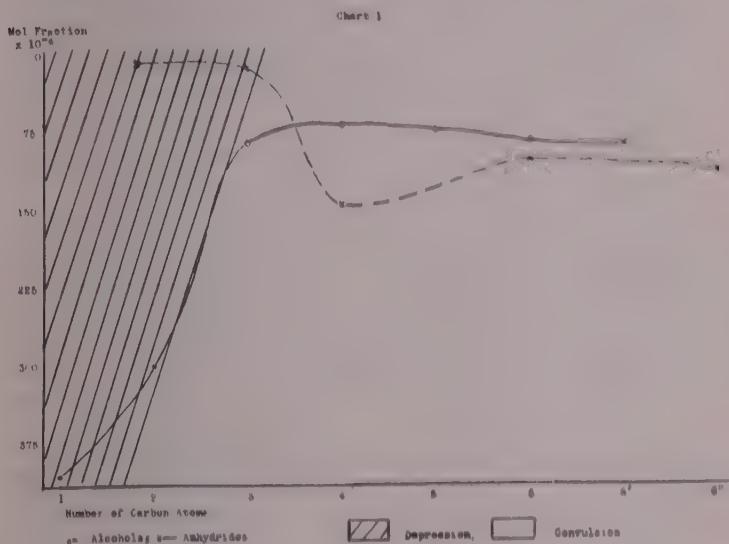
⁴ Beck, F. F., Master's Thesis, University of Maryland, 1930.

⁵ Dozois, K. P., Hachtel, F., Carr, C. J., and Krantz, J. C., Jr., *Am. J. Bact.*, 1935, **30**, 189.

period of 2 hours at $29^\circ \pm 1^\circ$ was considered the acute toxic dose. For each substance 15 to 30 mice were used.

The sugar alcohols of higher molecular weight and their anhydrides probably do not kill by specific action. They are toxic, possibly, only by virtue of the osmotic pressure changes which they induce, as the sugars, glucose and sucrose, are toxic in these concentrations.

A summary of the toxicities is shown in Chart 1.



Summary. A direct relationship exists between the molecular weight and the toxicity of the alcohols up to that with 4 carbon atoms; the relationship is inverse with their anhydrides.

The first 2 anhydrides are more toxic than their corresponding alcohols, while the anhydrides of the higher homologues are less toxic, or possess about the same degree of toxicity as their precursors.

Streptococcal Inhibition of Non-Specific Inflammatory Fixation.

E. W. DENNIS.

From the Department of Bacteriology and Parasitology, School of Medicine,
American University of Beirut, Beirut, Syria.

Dennis and Berberian¹ recently described the ability of certain strains of *Streptococcus hemolyticus* and *Streptococcus viridans* to inhibit (delay) the process of inflammatory fixation in the skin of the rabbit when *Staphylococcus aureus* whole culture was used as the irritant. Berkefeld filtrates, as well as the whole-cultures of streptococci exhibited the property of delaying inflammatory fixation as long as 4-6 hours. The experiments reported below were carried out with the hope that the inhibitory effect might be even more sharply defined if a substance such as aleuronat were used as the irritant, thus avoiding the multiplicity of factors which must be active in the antagonistic association of streptococci and staphylococci as in our earlier experiments. Menkin² has previously reported that the injection of a suspension of aleuronat into the skin of the rabbit induced the inflammatory fixation of trypan blue with in a period as short as 30 minutes.

Can *Streptococcus hemolyticus* whole-culture inhibit or delay the inflammatory fixation normally elicited by aleuronat in the skin of the rabbit?

Aleuronat (5%) was suspended in a 3% solution of soluble starch in plain broth. The suspension was autoclaved at 10 lbs. pressure for 10 minutes, and thoroughly agitated every few minutes while cooling. After standing for several hours the upper portion consisted of a uniform suspension of finely divided particles suitable for injection through a 27G needle.

Two series of rabbits, Nos. 41-45, and 46-50 inclusive were used in the experiment. Nos. 41-45 each received 0.5 cc. of 18 hr. whole-culture of *Strep. hemolyticus* (strain "Gay") grown in dextrose phosphate broth, plus 0.5 cc. of aleuronat suspension intradermally into the right foreleg; the culture and aleuronat suspension were mixed in the syringe. 0.5 cc. of aleuronat suspension plus 0.5 cc. of dextrose phosphate broth adjusted to pH 6 (pH of 18 hr. whole culture used) were mixed in the syringe and injected into the skin of the left foreleg of each rabbit as the control. Rabbits 46-50

¹ Dennis, E. W., and Berberian, D., *J. Exp. Med.*, 1934, **60**, 581.

² Menkin, V., *Ibid.*, 1929, **50**, 171.

were treated in an identical manner except that strain S 23M of *Strep. hemolyticus* was employed for the injections into the right foreleg; the left foreleg was used for the control injection. The degree of inflammatory fixation was determined at intervals of 2, 4, 6, 8, and 24 hours after the injection of the aleuronat and aleuronat streptococcus mixture by injecting 0.8 cc. of 1% solution trypan blue in saline into the inflamed skin of both forelegs of the rabbits to be examined at that period. Exactly one hour after the injection of the dye into a rabbit, the animal was placed under ether anesthesia and the regional lymph nodes in the axilla examined for the presence of the dye. The inflamed areas of skin were removed and fixed in formalin for subsequent histological examination.

The results are presented in Table I. The degree of discoloration of the lymph nodes, indicative of the degree of patency of the lymphatics and absence of the inflammatory fibrinous barrier (controlled by microscopic examination of stained sections) in the area of local inflammation is indicated by an appropriate number of plus signs. Conversely, inflammatory fixation is indicated by the failure of the dye to reach the lymph nodes in significant quantity. As shown in Table I, aleuronat suspension alone promptly produced

TABLE I.
Inhibition of Nonspecific Inflammatory Fixation by Streptococci.

| Rabbit No. | Duration of inflammation hrs. | Passage of dye to lymph nodes | | | |
|---------------|-------------------------------------|-------------------------------|-------|-------|-------|
| | | Right* | | Left† | |
| | | S (a) | D (b) | S (a) | D (b) |
| 4-1 | 2 | +++ | +++ | + | - |
| 4-2 | 4 | ++ | ++ | + | ± |
| 4-3 | 6 | +++ | +++ | ± | - |
| 4-4 | 8 | +++ | ++ | - | - |
| 4-5 | 24 | ++ | + | - | - |
| 4-6 | 2 | ++++ | +++ | ± | - |
| 4-7 | 4 | ++ | +++ | ++ | - |
| 4-8 | 6 | ++++ | +++ | + | ± |
| 4-9 | 8 | +++ | ++ | + | - |
| 5-0 | 24 | ++ | + | ± | ± |

*Aleuronat plus streptococcus whole culture injected into right foreleg. Nos. 4-1 to 4-5 received strain "G"; Nos. 4-6 to 5-0 received strain S 23 M.

†Aleuronat plus plain broth injected into left foreleg.

The number of plus signs indicates the relative amount of trypan blue which passed from the site of inflammation to the regional (axillary) lymph nodes at given periods of time after the injection of the aleuronat and aleuronat-streptococcus mixtures. The presence of the dye in the nodes indicates lack of inflammatory fixation and inadequacy of the inflammatory barrier; absence of the dye indicates the establishment of an efficient barrier.

(a) S = superficial lymphnodes of the injected region.

(b) D = Deep lymphnodes of the injected region.

fixation of the dye (within 2 hours). In the presence of *Streptococcus hemolyticus* whole-culture, inflammatory fixation, i. e., barrier formation was delayed for at least 24 hours.

Conclusion. *Streptococcus hemolyticus* whole-culture is capable of inhibiting the inflammatory fixation which is normally elicited by aleuronat in the skin of the rabbit.

It has been clearly demonstrated that the dissemination of streptococci, staphylococci, and *Pseudomonas aeruginosa* from an inflammatory focus in the skin to the regional lymphnodes and thence to the blood stream is largely governed by the efficiency of the fibrinous barrier which is responsible for what Menkin has termed "inflammatory fixation".^{1, 2, 3} It appears to be quite clear that the necrotizing property of *Staphylococcus aureus* is responsible for the characteristic localized nature of staphylococcal infections, by eliciting a prompt and intense inflammatory response.^{3, 4} Menkin^{2, 5} has suggested and maintained that *Streptococcus hemolyticus* paradoxically owes its characteristic invasiveness to a lack of irritating properties and consequent delay in eliciting an inflammatory response. The experimental data given by Dennis and Berberian¹ and the clearly defined results given above indicate that one need not resort to a paradoxical interpretation of the observed phenomena. If *Streptococcus hemolyticus* is capable of inhibiting the formation of an inflammatory barrier in the presence of an irritating substance such as aleuronat, it seems not unreasonable to suppose that this ability to interfere with the normal inflammatory response may play an important rôle in the dissemination of streptococci from a portal of entry under natural conditions.

² Menkin, V., *Ibid.*, 1933, **57**, 977.

⁴ Menkin, V., and Walston, H. D., *PROC. SOC. EXP. BIOL. AND MED.*, 1935, **32**, 1259.

⁵ Menkin, V., *Am. J. Med. Sci.*, **190**, 583.

8873 C

**Influence of Specific Antiserum on the Inflammatory Fixation of
Streptococcus hemolyticus.**

E. W. DENNIS.

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 American University of Beirut, Beirut, Lebanon.*

It has been demonstrated^{1, 2} that the formation of the inflammatory barrier and consequent "inflammatory fixation" is greatly delayed when invasive strains of hemolytic streptococci are inoculated into the skin of rabbits. The delayed fixation, in the case of *Streptococcus hemolyticus*, is due to the ability of this organism to actively suppress the formation of the inflammatory barrier to a considerable degree, as demonstrated by the ability to delay fixation even in the presence of such inflammatory irritants as *Staphylococcus aureus*,² and aleuronat.³ Further experiments have been carried out to determine the influence of streptococcal antiserum on the fixation time in local streptococcal inflammation.

Will streptococcus antiserum induce inflammatory fixation of *Streptococcus hemolyticus*?

Five cc. of "Streptoserin" (Bayer) were added to 5 cc. of a fresh 16-hour dextrose phosphate infusion broth culture of *Streptococcus hemolyticus*, and 5 cc. of fresh normal horse serum were added to 5 cc. of culture for use as a control. The culture-serum mixtures were allowed to stand at room temperature for 30 minutes before use. One cc. of the antiserum-streptococcus mixture was injected into the skin of the right foreleg of each of eight rabbits; 1 cc. of the normal horse serum-streptococcus mixture was similarly injected into the skin of the left foreleg of each rabbit. The degree of inflammatory fixation was subsequently determined at regular intervals by injecting 0.8 cc. of 1% trypan blue solution into the center of each inflamed area and examining the regional lymph nodes for the presence of the dye 1 hour after its introduction into the area of inflammation. The inflamed areas of skin were fixed in Zenker-formol and subsequently studied microscopically.

The results of the experiment are shown in Table I. Complete fixation occurred in the presence of specific antiserum in less than 2 hours. On the control side the dye passed freely to the regional

¹ Menkin, V., *J. Exp. Med.*, 1933, **50**, 977.

² Dennis, E. W., and Berberian, D., *Ibid.*, 1934, **60**, 581.

³ Dennis, E. W., *Proc. Soc. Exp. Biol. and Med.*, 1936, **35**, 100.

lymph nodes, indicating lack of fixation and inadequacy of the inflammatory barrier, for at least 28 hours. The presence of a fibrinous barrier in the area which retained the dye was confirmed by histological study.

TABLE I.
Influence of Streptococcus Antisera on Inflammatory Fixation in Acute Local Streptococcal Inflammation.

| Rabbit No. | Duration of inflammation, hr. | Passage of dye to lymph nodes | | | |
|---------------|-------------------------------------|-------------------------------|-------|-------|-------|
| | | Right* | | Left† | |
| | | S (a) | D (b) | S (a) | D (b) |
| 7-0 | 1 | ++++ | — | +++ | — |
| 7-1 | 2 | — | — | ++++ | +++ |
| 7-2 | 4 | — | — | +++ | +++ |
| 7-3 | 6 | — | — | +++ | +++ |
| 7-4 | 8½ | — | — | +++ | +++ |
| 7-5 | 10 | — | — | +++ | +++ |
| 7-6 | 22 | ± | — | +++ | +++ |
| 7-7 | 28 | ± | — | +++ | ++ |

The number of plus signs indicates the relative amount of trypan blue which passed from the site of inflammation to the regional (axillary) lymph nodes at given periods of time after the injection of the streptococcus-serum mixtures. The presence of the dye in the nodes indicates lack of fixation and inadequacy of the inflammatory barrier; absence of the dye indicates the establishment of an efficient barrier.

*Received *Strep. hemolyticus* plus streptococcus antiserum.

†Received *Strep. hemolyticus* plus normal horse serum (control).

(a) S = superficial lymph nodes of the region of injection.

(b) D = deep lymph nodes of the region of injection.

Conclusion. Inflammatory fixation is promptly induced in acute local streptococcus inflammation in the presence of streptococcus antiserum, in contrast with the marked delay in fixation in the presence of normal serum.

The experiment presented above has been repeated on several occasions. Five different strains of *Streptococcus hemolyticus*, and 4 different commercial brands of polyvalent so-called antitoxic-antibacterial streptococcus antisera have been used with essentially identical results.

The data given above suggest that the factor produced by *Streptococcus hemolyticus* which is responsible for the active suppression of the formation of the local inflammatory barrier is antigenic and is readily neutralized by streptococcus antiserum. A possible alternative explanation is that the inflammatory fixation is part of an Arthus phenomenon due to the introduction of a mixture of antigen and antibody into the skin. However, in view of the ability of the "inhibitory factor" to suppress fixation in other types of inflammatory response, this explanation would seem to be improbable,

and attempts to test the hypothesis experimentally have given negative results.

Ward and Lyons^{4, 5} have shown that the virulence of hemolytic streptococci is ultimately dependent upon the resistance of the organism to phagocytosis. However, invasiveness (*i. e.*, the ability of the organism to disseminate from the portal of entry) is a vitally important corollary of virulence. We have found that avirulent strains of streptococci may rapidly reach the blood stream following injection into the skin, but are quickly disposed of by the cellular defenses of the body; on the other hand the speed with which organisms which are resistant to phagocytosis kill the host may be determined by the ease with which they reach the blood stream. Menkin⁶ has recently demonstrated that the early establishment of a nonspecific inflammatory barrier at the site of injection into the skin may protect rabbits against otherwise lethal doses of pneumococci known to be highly resistant to phagocytosis.

Thus it would seem that, despite the general lack of specific opsonins in commercial streptococcus antisera, the local introduction of adequate amounts of such antisera directly into the involved area of an acute local streptococcal inflammation, would offer a means of facilitating or maintaining the localization of the infectious process by the enhancement of the fibrinous barrier and "inflammatory fixation".

8874 P

Sorbitol as a Diuretic.

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The hexahydric alcohol, sorbitol, has recently become available in quantities at a low price. It is non-toxic, has enormous water binding capacity, is rapidly excreted by the kidneys after intravenous injection and has 1.88 times the osmotic pressure of the same percentage sucrose solution. Its solutions are less viscous and more easily injected than those of sucrose and are entirely stable to

⁴ Ward, H. K., and Lyons, C., *J. Exp. Med.*, 1935, **61**, 515.

⁵ Lyons, C., and Ward, H. K., *Ibid.*, 1935, **61**, 531.

⁶ Menkin, V., *J. Infect. Dis.*, 1936, **58**, 81.

heat sterilization. It apparently possesses all of the properties desirable in a physical diuretic.

A study of the diuretic action of a 50% sorbitol solution (prepared from the syrup supplied by Atlas Powder Company) as compared with that of a 50% sucrose solution has been made in 3 dogs. The dogs were placed under nembutal anesthesia and kymographic records made of arterial pressure, respiration, and urine flow from a catheter placed in the bladder. Urine volumes were measured. Fig. 1 shows the rates of urine excretion (volume per 15-minute period) obtained after intravenous injection of 50 ml. of 50% solutions of sucrose and sorbitol. The curves are numbered in the order of injection. When injected at the rate of 50 ml. in 2 minutes ($38^{\circ}\text{C}.$) neither sucrose nor sorbitol caused appreciable changes in blood pressure or respiration. The curves indicate sorbitol to be a much more efficient diuretic in dogs than sucrose.

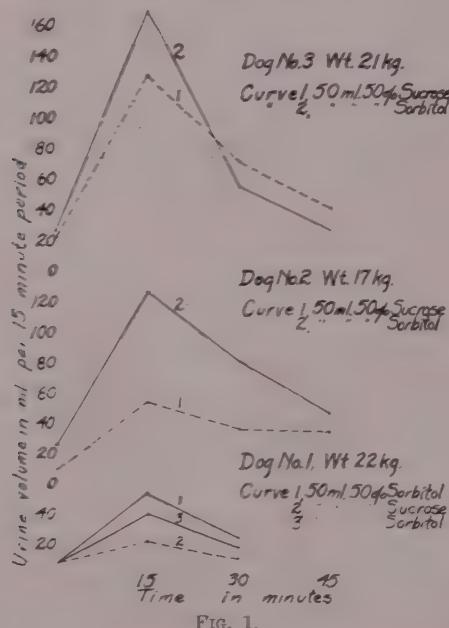


FIG. 1.

In the case of Dog 1, sorbitol was injected first (Curve 1), followed by sucrose (Curve 2), and then a second injection of sorbitol was given. No replacement of body fluids during the experiment excepting the water given with the solutions injected. Curve 3 shows that sorbitol had a much more powerful diuretic action than

sucrose even after the dehydration produced by the previous injections. In the case of Dog 2, 100 ml. of Ringer's solution was given before both sucrose and sorbitol. Otherwise procedure as in Dog 1. In the experiment on Dog 3 Ringer's solution was injected before both sucrose and sorbitol in quantity to compensate for fluid lost.

In view of the apparent superior diuretic action of sorbitol, it would seem to hold promise of being a valuable clinical diuretic agent. Its possibilities are being further investigated.

8875 P

Utilization of the Arsenic Analogue of Choline Chloride in the Bio-Synthesis of Phospholipid.

ARNOLD DEM. WELCH. (Introduced by C. F. Cori.)

From the Department of Pharmacology, Washington University School of Medicine, St. Louis.

The mechanism by which choline chloride influences the deposition of fat in the liver is by no means clear. Best, Channon and others have shown that this nitrogenous component of lecithin is capable of preventing or curing the fatty infiltration of the liver which is produced in the rat on a high fat diet or in the dog on a lean meat-sucrose diet. The finding that diets essentially free of choline cause an infiltration of fat into the livers of rats, a condition curable by the addition of choline chloride to the diets, led Best, *et al.*, to suggest that choline may be an essential dietary factor.

The most obvious hypothesis as regards the manner in which choline produces its "lipotropic" effect would involve perhaps the formation of lecithins and other choline-containing phospholipids from fat, phosphate and ingested choline, thus favoring the transport of lipid materials. There are obstacles to the immediate acceptance of such a view, of possible significance among which is the lipotropic inactivity of aminoethanol, the nitrogenous constituent of the phospholipid cephalin. Of greater import is the finding (Best, *et al.*) that betaine, the naturally occurring acid corresponding to choline, is lipotropically active. This substance is incapable of entering into the formation of phospholipids unless it is first reduced to choline by the organism, a conversion which would be of considerable biochemical interest.

Pharmacological investigations of the phosphorus and arsenic

analogues of choline and acetylcholine,^{1, 2} molecules in which arsenic replaces nitrogen as the nuclear element, suggested that the arsenic analogue of choline chloride ("arsenocholine" chloride) might prove useful in furnishing essentially a "tagged molecule," the assimilation of which by the organism might be followed.

It has been found that arsenocholine chloride[†] exerts no apparent toxic effects in the doses employed and is approximately as effective as choline chloride in preventing the fatty changes produced in the livers of rats on a high fat-low choline diet.[‡] That the organism utilizes the arsenic analogue in the biological synthesis of lecithin is indicated by the following: A group of rats was fed a high fat-low choline diet, containing sufficient arsenocholine chloride to supply an average of 17 mg. per 100 gm. rat per day (equivalent to 11.8 mg. of choline chloride and containing 6.4 mg. of arsenic). After a period of 21 days on this diet, lecithin, in the form of the purified cadmium chloride complex,[§] was isolated from the brains and livers of the rats. Through the kindness of Dr. Gordon H. Scott, of the Department of Anatomy, carbon arc spectrograms of the materials were obtained and found to contain strong arsenic lines, while no such lines could be demonstrated in the spectrograms of lecithin similarly prepared from control animals.

Less intense but definite arsenic lines were seen in the purified mercuric chloride complex of sphingosine-phosphoryl-choline isolated, according to the technic of Booth,[¶] from the kidneys of rats on a diet containing arsenocholine chloride.

Such tagging of phospholipid molecules suggests many applications in the study of phospholipid metabolism and in histochemical studies of cellular structures of a known or suspected phospholipid nature.

The mechanism of the lipotropic action of choline and betaine may possibly be approached through the administration of the arsenic analogue of betaine chloride; subsequent isolation of arsenic-containing phospholipids would evidence the ability of the organism to convert betaine to choline and support the hypothesis which suggests that these substances function lipotropically through the conversion of fat to phospholipid. Experiments of this nature are now under way.

¹ Welch, A. DeM., and Roepke, M. H., *J. Pharmacol.*, 1935, **55**, 118.

² Roepke, M. H., and Welch, A. DeM., *J. Pharmacol.*, 1936, **56**, 319.

[†] The arsenocholine chloride was generously supplied by F. Hoffmann-La Roche and Co.

[‡] Yeast concentrate kindly supplied by E. R. Squibbs and Sons.

[§] Levene, P. A., and Rolf, I. P., *J. Biol. Chem.*, 1927, **72**, 587.

[¶] Booth, F. J., *Biochem. J.*, 1935, **29**, 2071.

Suitability of the Monkey (*Macacus rhesus*) as a Recipient for the Prausnitz Kustner Reaction.

A. H. W. CAULFEILD, M. H. BROWN AND E. T. WATERS.

From the Allergy Clinic of one of us, the Connaught Laboratories, and the Department of Physiology, University of Toronto.

It has been shown that all guinea pigs injected with alum precipitated ragweed extract¹ developed an unusually high degree of sensitivity. Blood serum² drawn from guinea pigs so treated has been shown to specifically sensitize human skin sites in a manner comparable to the results obtained by the intradermal injection of human serum from a case of ragweed hay fever (Prausnitz Kustner Reaction).

With this observation (identity of antibody) it became increasingly desirable to determine whether any animal would give positive skin tests while in an anaphylactic state.

On previous occasions the intradermal injection of ragweed extract into actively sensitized guinea pigs and rabbits had always been negative with the guinea pigs and unconvincing in the rabbit.

In order to save the time required for active sensitization it was thought our objective—if it existed—might be accomplished more rapidly and easily by attempting to passively sensitize the skin by the intradermal injection of sera already proven to have skin sensitizing antibodies for human skin. The first animal tried in this plan was the monkey (*Macacus rhesus*).

TABLE I.

Prausnitz-Kustner Reactions with Monkey 49.
 July 27, 1936—Sites injected with 0.05 cc. serum as noted below.
 July 28, 1936—All sites injected with 0.05 cc. ragweed extract 2036 with reactions as noted below and as illustrated in photographs.

| Site | Left Side | | Right Side | |
|---------------|------------------------------|-------------|------------------------------|----------|
| | Degree of reaction in mm. | Site | Degree of reaction in mm. | Site |
| 1. G.P.S. 503 | 10 x 12 | G.P.S. Nor. | | Negative |
| 2. Hu.S.Iv. | 15 x 22 | Hu.S. Nor. | | " |
| | 3. 0 control negative | | | |

Abbreviations used:

G.P.S. 503—Blood serum from sensitized guinea pig No. 503.

G.P.S. Nor.—" " " normal guinea pig.

Hu.S.Iv.—" " " a case of human ragweed hay fever (Iv.).

Hu.S. Nor.—" " " normal human.

0 control—normal skin site.

¹ Caulfeild, Brown and Waters, *J. Allergy*, 1936, **75**, 451.

² Caulfeild, Brown and Waters. In press.

Serum from sensitized guinea pig 503 was used in this experimental trial. With this serum we had been successful in sensitizing human skin sites, the full details of which were given previously.² The human serum (Iv) from a case of ragweed hay fever had been used for Prausnitz Kustner reactions many times previously. It was known to have a high titre.

The results are shown in Table I and in the accompanying photo-





tographs.* The reaction obtained in both sensitized guinea pig and human serum sites is clear cut and positive whereas in both normal guinea pig and human serum sites there is no reaction. The normal skin site control is also clearly negative.

* The wheal of a positive reaction in the monkey is more difficult to reproduce in a photograph than the areola of a positive reaction in man. We took 2 on this account. In one of these the camera was focused on the 2 positive reactions (on the left); in the other the camera was focused on the negative and control sites (on the right).

These positive reactions in locally sensitized monkey skin sites differ from positive reactions in man as the recipient for the Prausnitz-Küstner reaction. In man a positive result consists of a button-like wheal without pseudopods and a surrounding areola. In the monkey the wheal shows marked pseudopodia formation and no surrounding areola. Instead there is a vague blueish tinge about the wheal which photographs fail to record.

Conclusion. The skin of the monkey (*Macacus rhesus*) is suitable for local passive sensitization to ragweed pollen extract by the intradermal injection of either serum from a sensitized guinea pig or serum from a human case of ragweed hay fever.

8877 C

Vaso-Pressor Action of Extracts of Plasma of Normal Dogs and Dogs with Experimentally Produced Hypertension.

IRVINE H. PAGE.

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Marked chronic hypertension may be produced in dogs by compressing the renal arteries by means of silver clamps until moderate ischemia occurs (Goldblatt, Lynch, Hanzal and Summerville¹). Nervous impulses originating in the kidneys do not appear to participate in the genesis of this hypertension, for if the renal nerves are severed, clamping the renal artery is still effective in causing hypertension (Page²). It is not unreasonable, therefore, to suppose that a pressor substance is liberated by the kidneys as a result of ischemia which might be responsible for the hypertension. The object of this investigation is to ascertain, by means of a method with which we had previously had experience (Page³) whether this hypothesis could be supported with experimental evidence.

Carotid loops were prepared in dogs for measurement of arterial pressure by the Van Leersum method. After a control period of 2 months during which blood pressure was measured daily, silver clamps were placed on the renal arteries according to the method of Goldblatt, Lynch, Hanzal and Summerville.¹ Amytal injected intraperitoneally (60 mg. per kg.) was employed as anesthetic.

¹ Goldblatt, H., Lynch, J., Hanzal, R. F., and Summerville, *J. Expt. Med.*, 1934, **59**, 347.

² Page, I. H., *Am. J. Physiol.*, 1935, **112**, 166.

³ Page, I. H., *J. Expt. Med.*, 1935, **61**, 67.

Within 3 days the arterial pressure rose, often as high as 260 to 300 mm. of Hg. Blood (60 cc.) was taken by puncture of the femoral artery before and after the production of hypertension. Heparin was used as anticoagulant. Alcoholic extracts of the plasma were prepared as previously described.³ The pressor effect of the extracts was ascertained by intravenous injection into cats (2.5-3.5 kg.) anesthetized with ethyl urethane (6 cc. of a 25% solution per kilo). Blood pressure was recorded by means of a mercury manometer with a cannula in the carotid artery. Both vagus nerves were cut. The same volume of warm salt solution as that of the extract was always injected before the extract to be tested was injected. From one to 5 weeks after production of hypertension in 3 of the dogs, the hypophysis was removed (Page and Sweet⁴). Arterial pressure fell to normal. Blood samples were again obtained and extracts prepared and assayed for pressor activity.

Results. Extracts of dog's blood appear to differ slightly from those of human blood in that the pressor response when injected into cats is usually smaller. Both are characterized by the fact that the pressor response is dependent on the functional intactness of the central nervous system, for if the animal is pithed the extracts are inactive. Typical examples of results derived from study of the injection of extracts into 18 cats are presented (Table I).

If the same volume of warm salt solution as that of the extract produced a rise of more than 2-4 mm. of Hg. the experiment was abandoned, consequently the rises in arterial pressure recorded in the table are not volume effects.

These data show that rise in the cat's blood pressure is as liable to occur from extracts of plasma from dogs with normal blood pressure as those with marked hypertension. Hypophysectomy, although reducing the arterial pressure, did not alter the character of the response to the plasma extracts as assayed by this method.

The curves of the pressor action of plasma extracts resemble those obtained from human blood (Figs. 1 and 2). In Fig. 1 marked pressor action is shown by extracts of plasma of a dog rendered hypertensive compared with plasma from the same dog when arterial pressure was normal. The reverse is shown in Fig. 2, that is, extract from plasma of a dog with normal blood pressure was the more active. Since responses to the same extract are so irregular, not only in different animals but in the same animal at different periods in the experiment, it was necessary to test many extracts

⁴ Page, I. H., and Sweet, J. E., PROC. SOC. EXP. BIOL. AND MED., 1936, **34**, 260.

VASO-PRESSOR ACTION OF DOGS' BLOOD

TABLE I
Response of Cat's Blood Pressure to Injection of Extracts of Plasma from Dogs
with Normal and Elevated Blood Pressure.

| Cat No. | Dog No. | Dog's blood pressure, mm. of Hg. | Volume of Extract | Equivalent amt. of plasma | Rise in cat's blood pressure, mm. of Hg. |
|---------|---------|----------------------------------|-------------------|---------------------------|--|
| 219 | 87 | 110 | 5 | 10 | 44 |
| 219 | 87 | 110 | 5 | 10 | 36 |
| 220 | 87 | 110 | 5 | 10 | 14 |
| 220 | 87 | 280 | 5.5 | 11 | 14 |
| 220 | 87 | 210 | 5 | 10 | 14 |
| 226 | 87 | 210 | 5 | 10 | 6 |
| 227 | 87 | 210 | 5 | 10 | 18 |
| 214 | 86 | 140 | 5 | 10 | 0 |
| 214 | 86 | 140 | 5 | 10 | 0 |
| 214 | 86 | 230 | 5 | 10 | 16 |
| 214 | 86 | 230 | 5 | 10 | 22 |
| 218 | 86 | 204 | 5 | 10 | 8 |
| 218 | 86 | 204 | 5 | 10 | 4 |
| 218 | 86 | 204 | 5 | 10 | 6 |
| 226 | 86 | 240 | 5 | 10 | 0 |
| 218 | 84 | 116 | 5 | 10 | 2 |
| 218 | 84 | 116 | 8.5 | 17 | 4 |
| 218 | 84 | 200 | 5 | 10 | 8 |
| 218 | 84 | 200 | 9 | 18 | 10 |
| 229 | 84 | 210 | 10 | 22.5 | 0 |
| 229 | 84 | 140 | 10 | 24.5 | 0 |
| 229 | 84 | 160 | 10 | 22.0 | sl. fall Hypophysectomy |
| 221 | 82 | 150 | 5 | 10 | 10 |
| 221 | 82 | 218 | | 2 | 10 |
| 221 | 82 | 218 | 2.5 | 5 | 42 |
| 221 | 82 | 150 | 4.8 | 9.6 | 2 |
| 222 | 82 | 278 | 5 | 10 | -2 |
| 222 | 82 | 278 | 5 | 10 | 14 After cocaine sensitization |
| 130 | 82 | 110 | 9 | 26 | 4 Hypophysectomy |
| 215 | 83 | 240 | 5 | 5 | 16 |
| 222 | 83 | 114 | 5.5 | 11 | 2 |
| 222 | 83 | 212 | 5 | 5 | 2 |
| 223 | 83 | 114 | 5 | 5 | 8 |
| 223 | 83 | 212 | 5 | 5 | 12 |
| 224 | 83 | 114 | 2.4 | 4.8 | 10 |
| 224 | 83 | 212 | 2.0 | 4.0 | 12 |
| 231 | 83 | 200 | 6 | 16 | 6 Hypophysectomy |

on different animals. Such data have been secured. They demonstrate no correlation between the height of the blood pressure of the animal from which plasma was obtained and the pressor response in cats.

Pneumographic records show that during the injection of extract, respiration may be markedly slowed and become shallow temporarily. Asphyxia might be responsible for part of the initial rise in pressure but against this hypothesis are the two facts that



FIG. 1.

Effect of extract of plasma on arterial pressure of a cat. Uppermost line records respiration, second line down records the movements of the nictitating membrane, third line records arterial blood pressure, fourth line records time in 10-second intervals, and fifth line, the base line. 1. Salt solution 10 cc. 2. Extract (9 cc.) of 24 cc. of plasma from dog No. 61. Arterial pressure of this dog was 230 mm. of Hg.

there is no correlation between the inhibition of respiration and the rise in arterial pressure, and that removal of the adrenal glands does not prevent the rise. Then too, rise in pressure from asphyxia is ordinarily transient, while response from plasma extract is exceptionally prolonged (10 to 30 minutes).

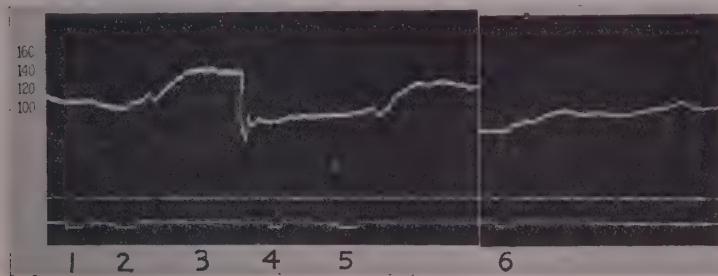


FIG. 2.

Effect of extract of plasma on arterial pressure of a cat. 1. Salt solution 5 cc. 2. Extract (5 cc.) of plasma of Dog No. 87; blood pressure 110 mm. of Hg. 3. Interval of 35 minutes. 4. Salt solution 5 cc. 5. Same as 2. 6. Extract (5 cc.) of 10 cc. plasma from dog No. 87 after renal artery was clamped. Blood pressure was 280 mm. of Hg.

These data do not support the hypothesis that hypertension produced by renal ischemia in dogs owes its genesis to circulation of peripherally acting pressor substances. They resemble those obtained by Page³ from study of plasma from patients suffering from hypertension associated with nephritis or of the essential type. In such cases, extracts of plasma also did not differ significantly from

extracts of plasma from normal persons in their action on cat's blood pressure.

It is of interest to observe that hypophysectomy in 3 animals did not alter the pressor action of the plasma extracts. It may be inferred that the hypophysis does not secrete its pressor element into the blood stream when hypertension is produced by clamping the renal artery in amounts large enough to be detected by the method. As yet there is no evidence to decide how important an effect its secretion may have on vaso-motor centers in the brain. It seems safe to conclude that the pressor principle ordinarily separated from pituitary glands is not present in the blood in sufficient quantities to cause hypertension in dogs by action on the peripheral blood vessels.

These results, in agreement with those of Prinzmetal, Friedman and Rosenthal⁵ obtained by transfusion of blood from normal to hypertensive dogs, suggest that some humoral substance is generated when the renal artery is constricted. While not itself directly pressor it causes abnormal constriction of blood vessels by some unknown mechanism.

Conclusion. Hypertension produced in dogs by means of compression of the renal artery has not been shown by the method employed to be caused by liberation of pressor substances into the blood stream which act directly on the peripheral blood vessels to cause vaso-constriction. Nor is the known pressor secretion of the pituitary gland present in amounts large enough to be detected by the method employed, in the blood of animals with hypertension. These conclusions are based on the following evidence: 1. Alcoholic extracts of plasma of dogs with hypertension when injected into anesthetized cats raises the cat's arterial pressure no more effectively than extracts from plasma of normal animals. 2. Removal of the hypophysis in animals with experimental hypertension does not alter the character of the pressor response to extracts of plasma.

⁵ Prinzmetal, M., Friedman, B., and Rosenthal, N., PROC. SOC. EXP. BIOL. AND MED., 1936, **34**, 545.

8878 P

Electrophoretic Anesthesia of Skin and its Application to Intra-dermal Testing in Hay-fever.

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Although the use of an electric field to force drugs into the skin has been known for many decades, surprisingly few experiments have been performed with local anesthetics. That the unbroken skin can be anesthetized by the electrophoresis of drugs into the skin has been known for many years, but this method has not been developed in the light of the modern theory of electrolytes.

It is of interest both theoretically in connection with electrokinetic properties of the skin, and practically in hay fever, to determine if the anesthetization of a small area is without essential influence on the development of moderately or markedly positive skin reactions.

By the use of an electrode especially designed to anesthetize a large number of small areas of the skin simultaneously, it has been possible to make intradermal tests with ragweed, timothy, dust, and other inhalants without any discomfort to the patient. Anesthesia lasting sufficiently long to perform skin tests can be obtained after 10 to 15 minutes electrophoresis of (1) procaine base in alcoholic or acetone solution, (2) butyn base, (3) cocaine base, (4) procaine hydrochloride, of various concentrations. Some effect was obtained with procaine borate. Agar jellies of the foregoing anesthetics were also effective. Control tests of allergens without the anesthetic have shown with few exceptions only the variability in skin reactions usually found on comparing 2 different skin sites. In fact, because of the anesthesia, the cooperation of the patient is more than usual and the fine needle may be inserted with greater care. Of the anesthetics employed, procaine base containing a trace of alkali has given the most consistent results with current densities of approximately 0.5 milliampere.

In a few instances, moderate or marked food reactions also have been compared. The anesthesia in the cases studied thus far has not decreased or enhanced these reactions although the most emphasis has been laid on experiments with inhalants.

Incidental to the anesthetization of the small areas required for intradermal tests, an erythema may arise. This erythema does not spread. The zone of anesthesia is in most instances definitely demarcated by this zone of erythema, although an erythema can be

produced by the current and the solvent alone. Because the method has not as yet been developed to the point where an erythema can be prevented, slightly positive reactions have not been investigated, although as mentioned above, both foods and inhalants have been successfully studied when moderate or marked reactions occur.

The skin can be anesthetized by procaine fast even in the presence of M. 30 NaOH. Under these circumstances, practically none of the positively charged form of the procaine is present. The undissociated base must, therefore, be carried passively by electroosmosis rather than by ionic migration. In support of this point of view, that the transfer of the procaine is electrolytic, wheals were produced when the histamine was dissolved in M. 30 NaOH. It can be shown that relatively few positively charged ions are present at the pH of this system, and that this is in accord with the point of view holding that electrophoresis as well as iontophoresis (the migration of the ion itself) may be employed to introduce drugs into the skin.

8879 P

Action of Certain Chlorinated Naphthalenes on the Liver.

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Three cases of yellow atrophy of the liver have occurred in each of 3 widely separated places and under different management within a year or two. In each instance where the illness appeared, the men were exposed to a chlorinated naphthalene heated above the melting point and giving off fumes. Yellow atrophy of the liver is a rare disease and there have been no cases reported where chlorinated naphthalene was the proven etiological agent.

The following experiments were conducted to determine if some of the chlorinated naphthalenes might produce such lesions. Three naphthalenes obtained from one of the above factories were used in this work. They are designated here as A, B, and C.

Compound A is a mixture of tri- and tetra-chlor-naphthalene. Compound B is a mixture of tetra- and penta-chlor-naphthalene and may contain some tri-chlor-naphthalene. Compound C is a mixture of penta- and hexa-chlor-naphthalene which is plasticized with a relatively small percentage of asphalt.

| Compound | Boiling Point | Melting Point | Chlorine Contents % |
|---------------------------------|---------------|---------------|------------------------|
| A | 600-650° F | 98-90° C | 47.9 |
| B | 615-655° F | 117.5-122° C | 49.8 |
| C | 680-730° F | 123-132° C | 47.9 |
| B sublimate given off at 192° C | " | 87-89° C | 35.3 |
| C " " " 172° C | " | 117-123° C | 53.1 |

(Sublimates given off by the naphthalenes were collected and injected into the animals under the same conditions as were the original compounds.)

Groups of 5 rabbits weighing approximately 2 kilos were used in testing each substance. Thirty milligrams of the compound dissolved in 2 cc. of paraffin oil were injected subcutaneously each day until the rabbit died.

None of the animals receiving injections of "A" died. The first death in the group receiving injections of "B" died on the 12th day and all were dead by the end of the 15th day. In the group receiving "C" the first rabbit died at the end of the 12th day and the last of the group on the 20th day. None of the rabbits receiving injections of sublimate from "B" died. The first death in the group receiving injections of sublimate from "C" died on the 9th day and the last one on the 14th day.

The animals affected showed no special symptoms outside of losing weight. The blood picture was not affected in either the red, white or differential count. The control animals, and animals receiving paraffin oil, "A" or sublimate from "B" gained in weight.

Autopsy Findings. Animals receiving "A" and sublimate B, when killed after 2 months injections, showed no pathological conditions that could be attributed to the exposure.

The following findings are typical of rabbits receiving "B", "C", and sublimate "C". There appears to be no reason for repeating the findings for each animal.

No. 533. Dosage: 30 mgr. of "C". 25 injections given.

Liver. Very large. Dark red with many opaque yellow areas. Capsule smooth. Cuts without resistance. Cut surface smooth. In many parts of the organ are yellow opaque areas with dull surface, that involve groups of lobules. In between, the liver parenchyma in places is dark red, and the lobules are clearly visible. In other places, the lobulation cannot be seen, the parenchyma has apparently disappeared and stroma is collapsed; these portions are greyish red. The gall bladder is distended.

In peritoneal cavity a moderate excess of bloody fluid.

Histologic Findings. Liver. Presents a most striking change. Beneath the capsule on one of the sections is a wide zone of necrosis, in which portal areas are seen surrounded by a single row of well preserved cells, or none at all. In other places in the section efferent veins are surrounded by wide zones in which liver cells have under-

gone necrosis. There are numerous other places where liver cells have completely disappeared and only the collapsed stroma remains. In many places calcification of the stroma is well advanced and larger masses of calcium are frequently seen. Multinucleated giant cells of huge dimensions are often clustered about the masses of calcium or are found in the areas of collapsed stroma.

Liver cells remain but the regular normal architecture is not retained. Rounder groups of liver cells are numerous or only narrow bands of these cells surround portal areas. The liver cells also present a peculiar arrangement in many places. A double column of the cells will open up leaving a considerable space surrounded by the cells. The space contains granular precipitate or large plugs of bile. We have the impression that these spaces may be hugely distended bile canaliculi, because they in some instances contain bile. Necrosis of individual liver cells is also taking place. Cirrhosis negative.

From the evidence obtained from 30 rabbits, all showing the same pathological picture we feel that certain chlorinated naphthalenes or impurities contained in them are capable of producing yellow atrophy of the liver in the rabbit. This, with the history of the industrial cases points to its being a possible etiological agent in the factory cases. No other material used in the factory was found to produce the lesion.

8880 P

Formation of Sulfide by Some Sulfur Bacteria.*

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Previous studies with the strictly autotrophic sulfur bacterium, *Thiobacillus thiooxidans*, growing on elemental sulfur have shown that the sulfur is rapidly oxidized to sulfate without the accumulation of intermediate products.¹ The strictly autotrophic sulfur bacterium *Th. thioparus* transforms thiosulfate to the 2 products, sulfate and elemental sulfur.² No question has arisen concerning the initial stages of transformation of thiosulfate, but, by reason of the relatively large size of the particles of elemental sulfur com-

* Journal Series paper of the New Jersey Agricultural Experiment Station, Department of Soil Chemistry and Bacteriology.

¹ Weissman, S. A., and Starkey, R. L., *J. Gen. Physiol.*, 1923, **5**, 285.

² Starkey, R. L., *J. Gen. Physiol.*, 1935, **18**, 325.

pared to the tiny bacterial cells (about $0.5 \times 0.8\mu$) it has been suggested that some initial transformation to a reduced or oxidized substance may precede passage of the sulfur material through the cell membrane, after which the reactions leading to the release of energy for growth takes place.³ However, McCallan and Wilcoxon present evidence which shows that the vapor pressure of elemental sulfur is sufficiently high to permit sulfur vapor to enter cells which are not even in contact with the solid sulfur.⁴

Inorganic media containing sulfur or thiosulfate and supporting growth of these 2 bacteria have been examined for the presence of sulfide. No sulfide or other reducing substance was detected in cultures of *Th. thioö.vidans* by titration with 0.01N iodine. No substance formed from thiosulfate by *Th. thioparus* was found by titration with iodine. Tests for sulfide with nitroprusside were negative in both cases. The results do not support the contention of von Deines that the sulfur material precipitated by *Th. thioparus* during growth on thiosulfate is a highly sulfured polysulfide.⁵

Although sulfide does not appear in detectable amounts in the media, sulfide is produced by both organisms during growth. Lead acetate paper suspended over the culture solution containing elemental sulfur and inoculated with *Th. thioö.vidans* showed slight darkening after growth for 10 days. The test for sulfide became increasingly stronger with longer growth. Cultures of *Th. thioparus* growing on a thiosulfate medium produced slight darkening of the lead-acetate paper in 4 days and within another week showed deep blackening. The test for sulfide was preceded many hours by the precipitation of sulfur in the medium, a transformation which characterizes growth of this bacterium on thiosulfate.

There can be no question as to the production of sulfide by both bacteria, but there is no indication that any appreciable quantity persists in the medium. It seems most probable that the sulfide is formed in both cases by reduction of elemental sulfur. The reduction of elemental sulfur to sulfide is effected by compounds containing -SH groups in animal and plant tissues and by glutathione. The occurrence of -SH groups has been established for a great variety of tissues and organisms including many filamentous fungi and bacteria. In the present studies, cellular material of several organisms including bacteria, fungi, and actinomycetes has been found to form

³ Buchanan, R. E., and Fulmer, E. I., 1930. *Physiology and Biochemistry of Bacteria*, The Williams and Wilkins Co., Baltimore, v. 8, 197.

⁴ McCallan, S. E. A., and Wilcoxon, F., *Contr. from Boyce Thompson Inst.*, 1931, **3**, 18.

⁵ von Deines, O., *Die Naturwissen*, 1933, **21**, 873.

sulfide from elemental sulfur. It is concluded that formation of sulfide by the sulfur bacteria is evidence that these organisms also contain substances possessing active -SH groups.

The fact that the sulfur bacteria hydrogenate sulfur during growth raises the question as to whether or not this is a necessary reaction preceding utilization of elemental sulfur as a source of energy. Should this prove to be the case it would necessitate a revision of conceptions concerning the mechanism of transformation of sulfur by these bacteria. It is equally possible that the sulfide may have no more significance in the nutrition of the sulfur bacteria than in that of the great number of heterotrophic microorganisms which have the same reducing capacity. By reason of the fact that the nature of sulfur precipitated by the sulfur bacteria belonging to the order Thiobacteriales is the same as that formed by *Th. thioparus*, and that the former may be presumed to contain compounds having -SH groups, there is reason to suspect that they are able to hydrogenate their sulfur globules and other added sulfur.

8881 P

Pressor Effects of Kidney Extracts from Patients and Dogs with Hypertension.

MYRON PRINZMETAL,* AND BEN FRIEDMAN.* (Introduced by B. S.
Oppenheimer.)

From the Mount Sinai Hospital.

A number of observers have found that extracts from the kidneys of various animals may have pressor effects.¹⁻⁴ The object of the present investigation was to determine whether extracts of kidneys from human beings and dogs with hypertension had greater pressor effects than those of control kidneys.

Kidneys were obtained at autopsy from 15 patients with hypertension and from 17 control subjects. The hypertensive group consisted of 9 patients with benign hypertension, 2 with malignant hypertension, 2 with chronic glomerular nephritis, and 2 with pyelonephritis and secondary contracted kidneys. The material was put

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¹ Tigerstedt, R., and Bergman, G., *Skand. Arch. f. Physiol.*, 1898, **8**, 223.

² Bingel, A., and Strauss, E., *Deutsch Arch. f. klin. Med.*, 1909, **96**, 476.

³ Léon, C., *Compt. med. Soc. de biol.*, 1898, **50**, 98.

⁴ Vincent, S., and Sheen, W., *J. Physiol.*, 1903, **29**, 242.

⁵ Pearce, R. M., *J. Exp. Med.*, 1909, **11**, 430.

⁶ Shaw, H. B., *Lancet*, 1906, **1**, 1295, 1375, 1455.

⁷ Forlanini and Riva-Rocci, quoted from Pearce.⁵

through a meat grinder, crushed in a mortar, shaken for 30 minutes with saline (one cc. per gm.), filtered and kept on ice. The tests were most satisfactorily performed on an unanesthetized dog with a Van Leersum (carotid) loop.⁸ In several instances records were obtained by direct cannulation of the carotid artery of anesthetized dogs and cats. Injections were made intravenously, using the equivalent of 7 gm. of tissue for the unanesthetized dog and smaller doses for the anesthetized animals.

A single injection usually resulted in a transient depressor effect followed by a gradual and prolonged rise which lasted from 10 minutes to over 3 hours. The average rise in blood pressure in the hypertensive group was 28 mm. of mercury, the range being 9 to 60 mm. of mercury. The average pressor effect in the non-hypertensive series was 12 mm. of mercury, the range being 0 to 48 mm. of mercury.

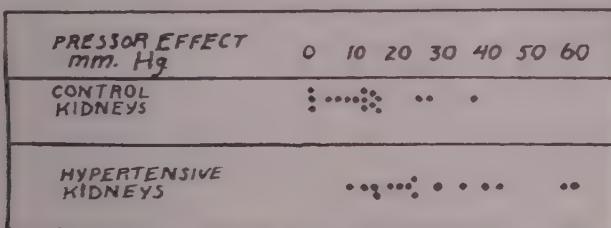


FIG. 1.

Pressor effects of saline extracts from kidneys of 15 patients with, and 17 subjects without hypertension.

The pressor action in general was more prolonged in the hypertensive than in the non-hypertensive group. No striking differences in the preliminary depressor effects were observed. In general the pressor effect seemed less marked in the younger age groups.

In 2 instances no rise in blood pressure was obtained. It is of interest to note that the blood pressures of the subjects were low in both cases (90 60-80 60). The pressor substance was more concentrated in the cortex than in the medulla and was completely destroyed by boiling for 5 minutes (one experiment). These properties suggest that it is identical with the substance found in normal kidneys by other workers.^{1,2}

In this small series no correlation was noted between the pressor effect on the one hand and the type and degree of hypertension, or the efficiency of renal function on the other.

It may be seen that considerable variation exists. This may be due, in part at least, to complicating factors, such as post mortem

⁸ Van Leersum, E. C., *Arch. Ges. Physiol.*, 1911, **142**, 377.

changes, manner of death, congestion and degeneration of the kidneys, age of patient, etc.

The above factors may be minimized by studying renal extracts of dogs with hypertension produced experimentally by means of unilateral renal ischemia.⁹ Fourteen such experiments were performed. After hypertension had been established, the ischemic kidney and the opposite unoperated kidney were removed, extracted and their pressor effects compared. In 11 out of 14 instances, the extract of the ischemic kidney had a significantly greater pressor effect than that of the normal kidney of the same animal, the average difference in maximum pressor effect being 23 mm. of mercury and the range 10 to 41 mm. of mercury. In 2 cases the differences were less than 10 mm. of mercury, and in one no difference was observed. In general the extracts made from ischemic kidneys appeared to have less pronounced preliminary depressor and more prolonged pressor effects than those prepared from normal kidneys. Harrison, Blalock and Mason¹⁰ have obtained similar results in dogs in which hypertension was produced by means of unilateral and bilateral renal ischemia, and by unilateral and bilateral ureteral obstruction.

The data are not sufficient to establish an etiologic relationship between the pressor substance in the kidneys and the elevation in blood pressure. The observations are compatible with the hypothesis that hypertension, both in the experimental animal and in man (whether "essential" or "secondary") may be due to an excess amount of a pressor substance present normally in the kidney.

8882 C

Positive Formol-Gel Reaction Associated with Hyperglobulinemia in Lymphogranuloma Inguinale, Multiple Myeloma and Hepatic Cirrhosis.

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It has been shown recently^{1, 2} that many cases of lymphogranuloma inguinale develop hyperproteinemia, comparable in degree, in-

⁹ Goldblatt, H., Lynch, J., Hanzel, R. F., and Summerville, W. W., *J. Exp. Med.*, 1934, **59**, 346.

¹⁰ Harrison, T. R., Blalock, A., and Mason, M. F. Personal communication.

¹¹ Williams, R. D., and Gutman, A. B., *PROC. SOC. EXP. BIOL. AND MED.*, 1936, **34**, 91.

² Gutman, A. B., Gutman, E. B., Jillson, R., and Williams, R. D., *J. Clin. Invest.*, 1936, **15**, 475.

cidence and fractional distribution to that long known to occur in kala-azar. This observation, it has been pointed out, affords an explanation for certain peculiarities of the serum in lymphogranuloma inguinale—peculiarities which appear to be common to most sera with definite hyperglobulinemia, irrespective of etiology: 1. increased erythrocytic sedimentation rate¹; 2. anticomplementary properties of the serum in a significant proportion of cases²; 3. apparent discrepancy in acid base equivalence of the blood in that the sum of the determined acid-equivalents appears to exceed the total base.² A further property of the serum in lymphogranuloma inguinale, likewise ascribable to hyperglobulinemia, is described in the present study. It was found that the formol gel test, used extensively for the diagnosis of kala azar, is positive in cases of lymphogranuloma inguinale presenting hyperproteinemia.

The total protein of the serum was determined by difference on 1 cc. samples, the macro-Kjeldahl technic being employed for total N and Folin's method with Nesslerization for non-protein N. Partitions were carried out in duplicate on 0.5 cc. samples by Howe's method. The formol-gel test was performed as follows: to 1 cc. of serum in a test tube (8 mm. bore is a convenient size) add 2 drops of 30-40% formaldehyde solution, mix, and allow to stand at room temperature. Normal control sera showed no change in viscosity or transparency in 24 hours.

Results. The formol-gel test was carried out on the sera of 13 cases of lymphogranuloma inguinale, all Negresses with positive Frei reactions, of whom 12 were under treatment for rectal stricture. The Wassermann reaction was positive in Cases 4, 6, 8, and 11, was persistently anticomplementary in Case 5 and negative in the remaining patients. The results are recorded in Table I. In Cases 1 and 2, with total serum proteins of 10.7 and 9.9% respectively, a semi-opaque gel formed within 5 minutes after the addition of formol. In 7 cases with less marked hyperproteinemia, a definitely positive response was obtained within 2 hours in most instances, and within 24 hours in all instances. In 4 cases of lymphogranuloma inguinale with serum proteins within normal limits, the sera were unchanged 24 hours after addition of the formol, or showed questionable slight increase in viscosity or opalescence.

Included in Table I are the results of the formol-gel test applied to sera obtained from patients with multiple myeloma and with cirrho-

¹ Gutman, A. B., and Williams, R. D., *J. Clin. Invest.* (Proc.), 1936, **15**, 458.

126 FORMOL-GEL REACTION IN LYMPHOGRANULOMA INGUINALE

TABLE I.
Formol-gel reaction in 13 cases of lymphogranuloma inguinale (L.I.), 6 cases of multiple myeloma (M.M.), and 3 cases of cirrhosis of the liver (C.L.); with representative results on 2 control sera (N.S.).

| Case | Diag-nosis | Serum Proteins | | | Formol-gel reaction of serum | | | | | |
|------|------------|----------------|---------|------------|------------------------------|--------|---------|----------|--------|---------|
| | | Total Prof. % | Glob. % | Iguglob. % | Gelation* | | | Opacity† | | |
| | | | | | 1/12 hr. | 2 hrs. | 24 hrs. | 1/12 hr. | 8 hrs. | 24 hrs. |
| 1 | L.I. | 10.7 | 7.8 | 4.0 | +++ | +++ | +++ | ++ | +++++ | +++++ |
| 2 | L.I. | 9.9 | 6.3 | 2.3 | +++ | +++ | +++ | ++ | +++ | +++ |
| 3 | L.I. | 9.7 | 6.2 | 2.4 | +++ | +++ | +++ | ++ | +++ | +++ |
| 4 | L.I. | 9.5 | 6.1 | 2.3 | +++ | +++ | +++ | ++ | +++ | +++ |
| 5 | L.I. | 9.1 | 5.6 | 2.9 | +++ | +++ | +++ | ++ | +++ | +++ |
| 6 | L.I. | 8.7 | 5.3 | 1.7 | +++ | +++ | +++ | ++ | +++ | +++ |
| 7 | L.I. | 8.6 | 5.0 | 1.7 | +++ | +++ | +++ | ++ | +++ | +++ |
| 8 | L.I. | 8.6 | 4.7 | 1.7 | +++ | +++ | +++ | ++ | +++ | +++ |
| 9 | L.I. | 8.5 | 4.8 | 0.9 | --- | --- | --- | ++ | ++ | ++ |
| 10 | L.I. | 7.7 | 3.9 | 0.9 | --- | --- | --- | ++ | ++ | ++ |
| 11 | L.I. | 7.5 | 3.4 | 0.9 | --- | --- | --- | ++ | ++ | ++ |
| 12 | L.I. | 7.3 | 3.1 | 0.7 | --- | --- | --- | ++ | ++ | ++ |
| 13 | L.I. | 7.0 | 3.3 | 1.1 | --- | --- | --- | ++ | ++ | ++ |
| 14 | M.M. | 11.1 | 8.8 | 0.4 | +++ | +++ | +++ | ++ | +++ | +++ |
| 15 | M.M. | 9.5 | 7.1 | 5.4 | +++ | +++ | +++ | ++ | +++ | +++ |
| 16 | M.M. | 9.2 | 6.1 | 2.3 | +++ | +++ | +++ | ++ | +++ | +++ |
| 17 | M.M. | 8.6 | 4.8 | 1.8 | +++ | +++ | +++ | ++ | +++ | +++ |
| 18 | M.M. | 7.7 | 2.3 | 0.4 | --- | --- | --- | ++ | ++ | ++ |
| 19 | M.M. | 6.3 | 3.2 | 0.6 | --- | --- | --- | ++ | ++ | ++ |
| 20 | C.L. | 8.4 | 6.0 | 2.1 | + | +++ | +++ | ++ | +++ | +++ |
| 21 | C.L. | 7.4 | 5.6 | 2.2 | --- | +++ | +++ | ++ | +++ | +++ |
| 22 | C.L. | 7.2 | 5.0 | 1.7 | --- | +++ | +++ | ++ | +++ | +++ |
| 23 | N.S. | 7.3 | 2.4 | 0.4 | --- | --- | --- | ++ | ++ | ++ |
| 24 | N.S. | 7.4 | 2.4 | 0.3 | --- | --- | --- | ++ | ++ | ++ |

*Gelation — no increase in viscosity
 ± questionable increase in viscosity
 + increased viscosity
 ++ very viscous flow
 +++ semi-solid
 ++++ solid

†Opacity — no change
 ± questionable slight opalescence
 + opalescent
 ++ translucent
 +++ semi-opaque
 ++++ opaque

sis of the liver. As reported by Sweigert⁴ and by Bing,⁵ an opaque gel forms rapidly in cases of multiple myeloma with marked hyperglobulinemia (Cases 14-17). A questionable slight opalescence after 24 hours was the only change noted in 2 additional instances with serum-proteins within normal limits.

Some patients with cirrhosis of the liver develop definite hyperglobulinemia. The serum of such patients gives a positive formol-gel reaction (Cases 20-22), the gel having a muddy-yellow appearance where jaundice is present (Cases 21 and 22). No change was noted within 24 hours in jaundiced sera without hyperglobulinemia.

Comment. 1. Opaque gel-formation has been described in few diseases other than kala-azar, chiefly in occasional cases of malaria, leprosy and tuberculosis,⁶ in all of which hyperglobulinemia may be marked. The formation of a *clear* gel, however, is said to occur sporadically in a variety of diseases,⁷ notably in syphilis.⁸ Opacity without gelation has been noted in early cases of kala-azar.⁹

The occurrence of gelation without opacity and opacity without gelation suggests that these two phenomena may be due to different factors. There is some experimental evidence that the formation of an *opaque* gel depends upon qualitative as well as quantitative changes in the serum-globulin fractions.^{9, 10} In accord with this view, we have found that *normal* serum, if it is sufficiently dehydrated, will gel after treatment with formol, but will not become opaque or opalescent. Normal serum concentrated *in vacuo* until the protein content was 18% congealed within 5 minutes; normal serum concentrated until the protein content was 12% formed a gel in 24 hours.* No opalescence developed after 48 hours. On the other hand, pathological serum of high protein content, which formed an opaque gel in 5 minutes, did not gel when the protein concentration was reduced to normal values by dilution with physiological saline solution. Opalescence, however, persisted even when the concentration of protein was reduced far below the normal level. Thus, the serum of Case 15 did not gel if diluted beyond 1:1½ with saline, but opalescence developed in all dilutions up to 1:10.

⁴ Sweigert, C. F., *Am. J. Med. Sc.*, 1935, **190**, 245.

⁵ Bing, J., *Acta med. Scand.*, 1936, **88**, 478.

⁶ Manson-Bahr, P. H., *Manson's Tropical Diseases*, London, 1935, 10th edition, p. 157.

⁷ Giraud, P., Montus, and Audier, *Bull. Soc. de Péd. Paris*, 1933, **31**, 115.

⁸ Gaté and Papacostas, *Compt. rend. Soc. de Biol.*, 1920, **83**, 1432.

⁹ Lloyd, R. B., and Paul, S. N., *Indian J. Med. Research*, 1928, **16**, 203.

¹⁰ Chopra, R. N., and Chaudhury, S. G., *Indian J. Med. Research*, 1929, **16**, 925.

* Gelation was not due to irreversible denaturation because after redilution to normal protein values, neither gelation nor opalescence occurred in 24 hours.

2. Case I-4 is of exceptional interest in that the increase in serum-globulin consistently involved only the pseudoglobulin 1 fraction as determined by Howe's method (*Cf.* Gros¹¹). The fact that this serum formed an opaque gel within 5 minutes after treatment with formol is contrary to the view that the formation of an opaque gel is dependent solely upon hypereuglobulinemia. Our data would suggest that a positive formol gel reaction is obtained in the presence of "abnormal" globulins irrespective of whether their solubility characteristics with respect to sodium sulfate correspond with those of euglobulin or pseudoglobulin.

3. We find the formol gel reaction a convenient preliminary test for the detection of gross hyperglobulinemia in lymphogranuloma inguinale. Its reliability for this purpose in other conditions is being investigated further.

8883 P

Experimental Production of Exophthalmos Resembling that Found in Graves Disease.

GEORGE K. SMELSER. (Introduced by P. E. Smith.)

From the Department of Ophthalmology, College of Physicians and Surgeons, Columbia University

Experimental production of exophthalmos has been accomplished repeatedly by stimulating the sympathetic innervation of the eye. MacCallum and Cornell,¹ Code² produced, by electrical stimulation of the cervical sympathetic ganglion of dogs and cats, a contraction of the smooth muscle elements in the orbit which caused exophthalmos. Schockaert,³ Loeb,⁴ Friedgood,⁵ and Marine^{6, 7} have reported exophthalmos resulting from the injection of thyrotropic anterior pituitary extracts into ducks, normal and thyroidectomized guinea pigs, and by the injection of methylecyanide into normal and thyroidectomized rabbits. Loeb and Schockaert noted that the exophthalmos

¹¹ Gros, W., *Deutsches Arch. f. klin. Med.*, 1935, **177**, 461.

¹ MacCallum, W. G., and Cornell, W. B., *Med. News*, 1904, **85**, 732.

² Code, C. F., and Essex, H. E., *Am. J. Oph.*, 1935, **18**, 1123.

³ Schocknert, J. A., *Am. J. Anat.*, 1932, **49**, 379.

⁴ Loeb, L., and Friedman, H., *PROC. SOC. EXP. BIOL. AND MED.*, 1932, **29**, 648.

⁵ Friedgood, H. B., *Bull. Johns Hopkins Hosp.*, 1934, **54**, 48.

⁶ Marine, D., and Rosen, S. H., *PROC. SOC. EXP. BIOL. AND MED.*, 1933, **30**, 901.

⁷ Marine, D., and Rosen, S. H., *Am. J. Med. Sci.*, 1934, **188**, 565.

produced was functional, *i. e.*, did not persist during anesthesia or after death. Marine showed that the protrusion resulting from injection of either pituitary or cyanide was accomplished through a nervous mechanism, presumably upon the unstriated orbital muscle of Müller, and that it could be prevented by removing the cervical sympathetic ganglion.

In the following experiment exophthalmos has been produced which does not depend upon a nervous mechanism but is due to an increase in orbital structures. It is readily obtained by the injection of beef anterior lobe extracts in thyroidectomized, but not normal, guinea pigs of both sexes. Twenty-six guinea pigs were injected with a thyreotropic anterior pituitary extract for 3 to 9 weeks with a daily dose equivalent of 250-2000 mg. of acetone dried beef anterior lobe powder. Loss in body weight and intense stimulation of the thyroid, as indicated by high epithelium, almost complete loss of colloid, and gross enlargement of 6-7 times, occurred. Of these only 3 showed slight indications of exophthalmos. Besides these questionable cases the eyes remained normal or became slightly enophthalmic.

The thyroids and left cervical sympathetic ganglion were removed from 26 guinea pigs which then received daily the equivalent of 250 mg. dry anterior lobe powder of the same preparation as the earlier experiment. Definite and, in a number of instances, extreme exophthalmos developed in all but 3 animals. Exophthalmos was usually noted 12-20 days after the first injection, although in one exophthalmos was not definitely established until 53 days after the first injection; it then became extreme.

In guinea pigs removal of the cervical sympathetic ganglion produces an enophthalmos and ptosis in the affected eye, and as a result exophthalmos produced in such eyes is less marked than it is in eyes with normal innervation, however, the protrusion is clearly demonstrated by comparison with the sympathectomized eye of a control.

The eyes of exophthalmic animals remain quite open postmortem, whereas those of normal guinea pigs partially or completely close. In many cases the sympathectomized eye is more protuberant than in life, a wide scleral band showing completely around the cornea.

The fat and connective tissue of the orbit appeared oedematous. The weights of the various orbital structures recorded at autopsy show that the retrobulbar tissue was increased to an average of 40% by these injections over controls consisting of normal, uninjected thyroidectomized, and thyroidectomized guinea pigs injected with extracts of thymus and liver. The control extracts were prepared in the same manner as the anterior pituitary and injected in a

daily dose equivalent to 1,000 mg. dry tissue, instead of 250 mg., for 30-62 days. An analysis of the autopsy data shows that the increase in the retrobulbar tissue was due to an increase of 100% in the fatty connective tissue, 40% in the dorsal lacrimal gland, and 22% in the extraocular muscles. It is somewhat doubtful if the ventral lacrimal is involved in this increase.

The structure of the orbital tissues, particularly the fat, was strikingly modified by an infiltration of a stainable material between the fat cells and collagenous fibers of connective tissue, so that the amount of fat is apparently reduced. This infiltrate which stains with eosin and anilin blue contains granules and droplets, presumably lipoid, as well as many lymphocytes scattered throughout the tissue. The infiltrate may be found, though to a lesser extent, between the lobules of the dorsal lacrimal and between the muscle fibres. Associated with the infiltration, in the muscles, nests of lymphocytes occurred in some cases. Small amounts of a material possessing staining properties similar to those of the infiltrate occurring in the exophthalmic animals may be found in the orbital fat of normal and thyroidectomized animals.

Because the increase in orbital tissue was as great in the eye from which the sympathetic innervation had been removed as in the normal, and since the postmortem exophthalmos was as great or greater in such eyes, it is concluded that an exophthalmos has been produced which is not dependent on a functional sympathetic innervation or the contraction of a smooth muscle, thus differing from the exophthalmos obtained by other experimental procedures.

Oedematous infiltration of the retrobulbar tissues has been repeatedly demonstrated in, and presented as the cause of, exophthalmos associated with Graves Disease. Such exophthalmos has been found associated with all ranges of B.M.R. and particularly extreme cases of progressive exophthalmos occur following thyroidectomy for exophthalmic goiter. Human biopsy specimens of muscles, orbital connective tissue, and fat obtained at operation from 2 low B.M.R. exophthalmos cases showed an infiltration indistinguishable from that obtained experimentally.

8884 C

Blood Inorganic Phosphates in Carbohydrate Metabolism.

M. PIJOAN AND T. B. QUIGLEY. (Introduced by E. C. Cutler.)

From the Laboratory of Surgical Research, Harvard Medical School, and Peter Bent Brigham Hospital.

Depression of the blood inorganic phosphates after the administration of dextrose has been demonstrated by Perlzweig, Latham, and Keefer.¹ This observation has been confirmed by Sokhey and Allen,² Harrop and Benedict,^{3, 4} and Bolliger and Hartman⁵ with both humans and animals receiving dextrose orally and parenterally. Although this fall in blood inorganic phosphates after the administration of dextrose appears to be the rule, it does not occur invariably. Blatherwick, Bell, and Hill⁶ in a series of experiments in which dextrose was given orally to human subjects, found no consistent change in the phosphate level and state that there is "sometimes an increase, or a decrease or no change at all."

Insulin, on the other hand, has been found by Wigglesworth, Woodrow, Smith and Winter⁷ to produce a marked drop in the plasma inorganic phosphate level of the rabbit. Cori and Cori,⁸ Perlzweig, Latham and Keefer,¹ and Vollmer⁹ have reported similar results, and further, have demonstrated that injections of epinephrin produce the same effect. Cori¹⁰ and Cori and Cori¹¹ in seeking an explanation for these changes found a marked increase in the hexose-phosphate content of the skeletal musculature of the normal animal after the injection of either insulin or epinephrin. No change in blood or muscle phosphates could be demonstrated in the adrenalectomized animal, and they concluded that the depression of the blood inorganic phosphate level following the injection of insulin

¹ Perlzweig, William A., Latham, E., and Keefer, C. S., *PROC. SOC. EXP. BIOL. AND MED.*, 1923, **21**, 33.

² Sokhey, S. S., and Allen, F. N., *Biochem. J.*, 1924, **18**, 1170.

³ Harrop, G. A., and Benedict, E. M., *PROC. SOC. EXP. BIOL. AND MED.*, 1923, **20**, 430.

⁴ Harrop, G. A., and Benedict, E. M., *J. Biol. Chem.*, 1924, **59**, 683.

⁵ Bolliger, A., and Hartman, F. W., *J. Biol. Chem.*, 1925, **64**, 91.

⁶ Blatherwick, N. R., Bell, M., and Hill, E., *J. Biol. Chem.*, 1924, **61**, 241.

⁷ Wigglesworth, V. B., Woodrow, C. E., Smith, W., and Winter, L. B., *J. Physiol.*, 1923, **57**, 447.

⁸ Cori, C. F., and Cori, G. T., *Arch. exp. Path. Pharmak.*, 1933, **172**, 249.

⁹ Vollmer, H., *Biochem. Z.*, 1923, **140**, 410.

¹⁰ Cori, C. F., *Physiol. Rev.*, 1931, **11**, 143.

¹¹ Cori, C. F., and Cori, G. T., *J. Biol. Chem.*, 1931-32, **94**, 581.

was the result of an increase in the secretion of epinephrin called forth by hypoglycemia. Ellsworth and Weinstein¹² found varying degrees of change in the blood inorganic phosphates in adrenalectomized dogs after the injection of insulin.

Thus, the mechanism of the drop in plasma inorganic phosphates after injection of insulin or epinephrin appears to be quite different from that following the administration of dextrose. Cori¹⁰ and Cori and Cori¹¹ have shown that after normal rats are fed dextrose there is no change in the hexose-phosphate content of muscle such as occurs following the injection of insulin or epinephrin. Furthermore, Pollock¹³ found that the parenteral administration of glucose produces the same fall in blood inorganic phosphates in normal and adrenalectomized animals.

In the literature on this subject little attention has been paid to the factors of diet and fasting. In this work dogs were used which had been fed a high-carbohydrate, low-fat diet (CHO 170, F 70, P 80) for at least 3 weeks before any experiment was carried out. Food was withheld from 8 such dogs for 20 hours, and their intake of water limited to 650 cc. each. They were then given one gm. of dextrose (Baker, C. P.) per kilo intravenously in 50% solution, and blood samples taken every 10 minutes for 2 hours. Blood sugar levels were determined by Folin's micromethod¹⁴ (using venous blood), and inorganic phosphates by the technic described by Fiske and Subbarow.¹⁵ This procedure was repeated at 3 intervals, after fasts of 10, 8, 4, and 2 hours. The results are about the same in the 8 dogs; for purposes of convenience only one is shown in the accompanying table. It will be seen that the depression of the blood inorganic phosphates varied directly with the duration of the fast beyond 8 hours.

The same experiment was repeated, substituting 1 unit of insulin (Mulford) per kilo for dextrose. Here the drop of the blood sugar seemed to depend to some extent on the length of the fasting period, while the drop in inorganic phosphates (average = 48% drop) was fairly constant, and was related to the point of maximal or "critical" hypoglycemia.

The adrenal glands were carefully removed in 2 stages from several of the dogs and 4 were maintained in sufficiently good condition for 3 weeks to allow the same studies to be made. The results are not in agreement with the Coris'⁸ hypothesis that the adrenals are

¹² Ellsworth, R., and Weinstein, A., *Bull. Johns Hopkins Hosp.*, 1933, **53**, 21.

¹³ Pollock, H., *Am. J. Physiol.*, 1933, **105**, 79.

¹⁴ Folin, O., *N. E. J. Med.*, 1932, **206**, 727.

¹⁵ Fiske, C. H., and Subbarow, Y., *J. Biol. Chem.*, 1925, **66**, 375.

TABLE I.
Effect of Fasting on Blood Glucose and Blood Inorganic Phosphates Following Administration of 1 gm. per kilo of Dextrose in the Normal Dog.

| Dog | Duration of fast, hr. | Fasting | | | 20 Min. | | | 40 Min. | | | 60 Min. | | | 80 Min. | | | 100 Min. | | | Maximum % Drop in Phosphates | |
|-----|-----------------------------|------------|------------|-----------------------|------------|------------|-----------------------|------------|------------|-----------------------|------------|------------|-----------------------|------------|------------|-----------------------|------------|------------|--|---------------------------------------|--|
| | | | | | | | | | | | | | | | | | | | | | |
| | | G mg. % | P mg. % | G P mg. % mg. % | G mg. % | P mg. % | G P mg. % mg. % | G mg. % | P mg. % | G P mg. % mg. % | G mg. % | P mg. % | G P mg. % mg. % | G mg. % | P mg. % | G P mg. % mg. % | G mg. % | P mg. % | | | |
| 1 | 2 | 102 | 5.4 | 242 | 5.6 | 194 | 5.0 | 182 | 5.1 | 164 | 5.2 | 141 | 5.3 | 7 | | | | | | | |
| | 4 | 100 | 5.6 | 250 | 4.9 | 181 | 4.8 | 160 | 4.7 | 148 | 4.8 | 132 | 5.0 | 16 | | | | | | | |
| | 8 | 102 | 5.6 | 200 | 5.0 | 184 | 4.6 | 152 | 4.5 | 141 | 4.8 | 128 | 4.8 | 20 | | | | | | | |
| | 10 | 86 | 5.8 | 196 | 4.8 | 178 | 4.6 | 138 | 4.0 | 120 | 4.1 | 118 | 4.0 | 31 | | | | | | | |
| | 15 | 82 | 5.6 | 210 | 4.8 | 180 | 4.1 | 140 | 3.6 | 132 | 3.6 | 110 | 3.6 | 35 | | | | | | | |
| | 20 | 80 | 5.8 | 230 | 4.6 | 161 | 4.0 | 137 | 3.8 | 123 | 3.2 | 100 | 3.4 | 45 | | | | | | | |

TABLE II.
Blood Glucose and Plasma Inorganic Phosphates in Adrenalectomized Pancrectomized Dogs Following 25 Units of Insulin Intravenously.

| Dog | Fasting | | | 10 Min. | | | 20 Min. | | | 40 Min. | | | 60 Min. | | | 70 Min. | | | | | |
|-----|------------|------------|-----------------------|------------|------------|-----------------------|------------|------------|-----------------------|------------|------------|-----------------------|------------|------------|-----------------------|------------|------------|--|--|--|--|
| | | | | | | | | | | | | | | | | | | | | | |
| | G mg. % | P mg. % | G P mg. % mg. % | G mg. % | P mg. % | G P mg. % mg. % | G mg. % | P mg. % | G P mg. % mg. % | G mg. % | P mg. % | G P mg. % mg. % | G mg. % | P mg. % | G P mg. % mg. % | G mg. % | P mg. % | | | | |
| 1 | 300 | 6. | 310 | 3.0 | 250 | 5.0 | 270 | 0 | 250 | 3. | 250 | 3. | 240 | 1. | | | | | | | |
| 2 | 140 | 8. | 170 | 4.1 | 120 | 4.3 | 90 | 4.6 | 110 | 5. | 110 | 5. | 110 | 4. | | | | | | | |

intimately associated with changes in the blood phosphate level during the metabolism of carbohydrates since a consistent, although irregular depression followed the injection of dextrose (as in Table I). On the other hand, their view that insulin acts on the blood inorganic phosphates through the adrenals is supported to some extent in that there was very little or no drop at all in these phosphates after adrenalectomy.

In view of the fact that insulin increases glycogen-storage in the diabetic animal, it was thought of interest to study its effect on the blood phosphorus in adrenalectomized animals with pancreatic diabetes. The pancreas and adrenals were removed from 2 dogs in 2 stages. About 5 hours after the second stage of the procedure (removal of the left adrenal) each animal had apparently completely recovered from the light ether anesthesia and was only slightly less than normally active. Twenty-five units of insulin were then given intravenously (Table II).

In these animals a marked fall in blood phosphates, and a moderate depression of the blood sugar level occurred. Both dogs died in about 8 hours after the second operation. Further work on this phase of the problem is in progress. Control animals showed a steady decline of the blood glucose as had been previously demonstrated, in the cat, by Long.¹⁶

The results, in general, suggest that the blood inorganic phosphates may be depressed by both glycogenesis and glycogenolysis. Thus, the fall in these phosphates may well be linked with the conversion of dextrose to glycogen in the liver, or glycogen to lactic acid in muscle. The ultimate fate of these phosphates in such processes has not as yet been revealed.

1. Starvation increases the degree of fall in blood inorganic phosphates occurring in normal dogs after intravenous administration of dextrose.
2. Removal of the adrenals does not influence the fall of blood inorganic phosphates following dextrose administration.
3. The injection of insulin has no effect on the level of blood inorganic phosphates after removal of the adrenals.
4. Removal of the pancreas and the adrenals accelerates the fall in inorganic phosphates, and decelerates the fall in blood sugar following the intravenous injection of insulin.

¹⁶ Long, C. N. H., *Ann. Int. Med.*, 1935, **9**, 166.

8885 C

Studies on Reduced Ascorbic Acid Content of the Blood Plasma.*

L. D. GREENBERG, J. F. RINEHART AND N. M. PHATAK.

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Recently Farmer and Abt¹ described a method for the determination of the reduced ascorbic acid in the blood plasma. Briefly, the procedure consists in deproteinizing the blood plasma with tungstic acid and titration with 2:6 dichlorphenolindophenol. They reported that the values obtained by this method parallel the intake of vitamin C and are an accurate index of the nutritional state relative to vitamin C. We have made many determinations of the reduced ascorbic acid in the blood plasma using essentially the technique of Farmer and Abt. We find it rapid and reliable and our data lead us to concur with their opinion of its significance.

It is the purpose of this communication to present briefly some of our own studies which indicate that the plasma level of reduced ascorbic acid is an accurate index of the immediate nutritive level with regard to vitamin C and in health parallels the intake of the vitamin. Equally convincing data in studies of rheumatic fever and rheumatoid arthritis is reported separately. With very few exceptions our study is based upon post absorptive blood specimens. All data presented refer to the ascorbic acid in the blood plasma present in the reduced form.

Influence of Small and Large Doses of Vitamin C upon Plasma Levels. The plasma ascorbic acid levels of 7 apparently normal adults were determined before and after ingestion of 6 oz. of orange juice. The results, which are summarized in Table I, show an appreciable rise of the plasma level 2 to 4½ hours after the administration of this relatively small dose of vitamin C. The increases ranged from 15% to 69%. For this reason we believe that fasting blood specimens are essential for comparative data.

In Fig. 1, the plasma ascorbic acid curves of 4 individuals who received large doses of ascorbic acid (0.5 gm. per 100 lb.) are shown. The curves are similar in type to those for blood sugar with

* This work was aided by a donation from the California Fruit Growers Exchange and by the Christine Breon Fund for Medical Research. We are indebted to Hoffmann-La Roche, Inc., for supplies of ascorbic acid.

¹ Farmer, C. J., and Abt, A. F., PROC. SOC. EXP. BIOL. AND MED., 1935, **32**, 1625; Abt, A. F., Farmer, C. J., and Epstein, I. M., J. Pediat., 1936, **8**, 1.

BLOOD PLASMA ASCORBIC ACID

TABLE I.
Increase in Ascorbic Acid of Blood Plasma After a 6-oz. Dose of Orange Juice.*

| Name | Plasma Ascorbic Acid | | Time After Ingestion of Orange Juice, hr. | Increase, Mg. % |
|----------|----------------------|-----------------|--|--------------------|
| | Before, Mg. % | After, Mg. % | | |
| L. L. G. | 0.94 | 1.04 | 2½ | .10 |
| | | 1.15 | 4 | .21 |
| L. L. G. | 1.01 | 1.39 | 4½ | .38 |
| A. H. | 0.77 | 1.07 | 2 | .30 |
| L. D. G. | 0.64 | 0.90 | 3¾ | .26 |
| J. F. R. | 1.12 | 1.29 | 4½ | .17 |
| P. N. | 0.54 | 0.82 | 4 | .28 |
| T. J. R. | 0.82 | 1.02 | 3½ | .20 |
| F. A. | 0.32 | 0.54 | 3½ | .22 |

* 6 oz. orange juice used by us contained on the average 108 mg. of vitamin C.

the peak of the curve occurring much later; *i. e.*, 2 to 4 hours after ingestion.

Plasma Levels in "Normal" Adults—Correlation with Urinary Excretion and Diet Habit. Determination of the post-absorptive plasma vitamin C concentration was made on 55 medical students. The levels ranged from 0.25 to 1.48 mg. %, with an average of 0.72 mg. %. Twenty-five, or approximately 45%, gave values below 0.70 mg. %, a figure which is considered to be the lower range of normal by Farmer and Abt.¹ Fasting specimens probably account

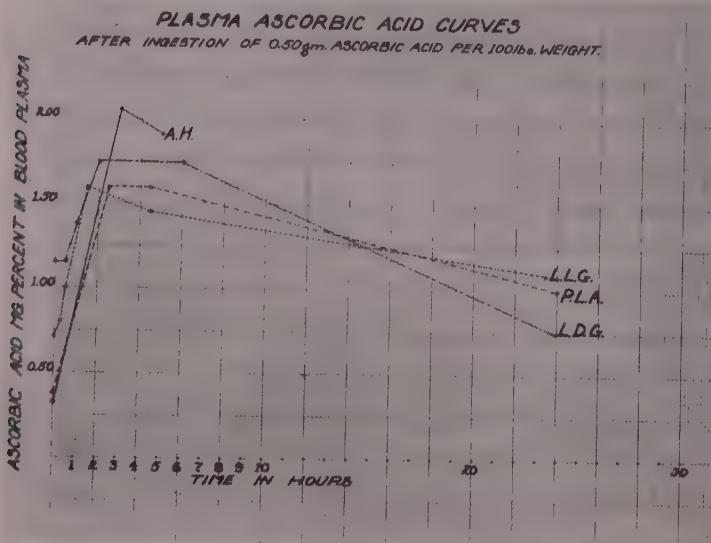


FIG. 1.

for lower levels in our series. It is also true that the nutritional habits of a rather large number of this group were inadequate. Using the test dose excretion procedure described by Harris and co-workers,² we find a close parallelism between the plasma ascorbic acid values and the urinary excretion following test doses of 250 to 300 mg. of vitamin C, either as orange juice or ascorbic acid. Of 25 students who excreted more than 50 mg. of ascorbic acid in the urine in the 24-hour period following the test dose, the average plasma value was 0.92 mg. %. The average value of 13 students who excreted less than 50 mg. was 0.44 mg. %. The recent dietary habit of 38 students was estimated with respect to vitamin C intake by an experienced nutritionist; 11 of these were rated as low in their vitamin C intake. In this group the average plasma ascorbic acid level was 0.55 mg. %. Of 19 cases considered fair in their vitamin C intake, the average plasma level was 0.75 mg. %. Eight students rated as good gave an average level of 1.1 mg. %. Complete data on this and a more extended "normal" group will be reported later. Seven students, who had low plasma vitamin C values initially, were given daily supplements of 100 mg. of ascorbic acid and subsequent determinations were made at various intervals. The results are summarized in Table II. It will be seen that 6 of the group responded with good or fair plasma concentrations of the vitamin in 5 to 7 days. The seventh student had a particularly poor nutritional history relative to vitamin C. Sixteen days were required to raise the plasma vitamin C to a correspondingly high level. These data clearly indicate a close parallelism between vitamin C intake and the value of reduced ascorbic acid in the blood plasma.

Further evidence is afforded in the following controlled dietary experiment on rhesus monkeys. Prior to beginning this experiment, 3 of the monkeys, (Nos. 1001, 1027 and 1032) had been main-

TABLE II.
Increase in Plasma Ascorbic Acid After Administration of 100 mg. of Vitamin C Daily.

| Name | Initial Plasma Ascorbic Acid, Mg. % | Duration of Treatment, Days | Final Plasma Ascorbic Acid, Mg. % |
|----------|---|-----------------------------------|---|
| J. D. H. | .46 | 5 | 1.06 |
| B. O. K. | .43 | 5 | 1.07 |
| Simard. | .42 | 11 | 0.80 |
| F. O. D. | .26 | 16 | 0.88 |
| | | 22 | 1.23 |
| J. N. C. | .31 | 7 | 0.78 |
| J. D. | .40 | 7 | 1.20 |
| L. L. H. | .58 | 7 | 0.86 |

² Harris, L. J., Ray, S. N., and Ward, A., *Biochem. J.*, 1933, **27**, 2011.

tained on a sub-optimal vitamin C intake (approximately 6 cc. orange juice daily) for 13 months. The fourth, a control animal (No. 1030), had received a daily supplement of one-half an orange during this same period. Plasma analyses for ascorbic acid were made during the week of January 10. The diets of 3 of the monkeys were then altered as follows: No. 1030 now received no supplement of vitamin C; No. 1027 and No. 1032 were given one-half an orange daily. Monkey No. 1001 was allowed to continue on an unchanged regime. Periodic determinations of the ascorbic acid of the plasma were carried out for 5 months. The plasma levels of the monkeys over this period of time are represented graphically in Fig. 2. Note the very prominent drop in curve of monkey No. 1030 after withdrawal of the supplement and again the rapid rise after restoring the supplement on May 14. In the case of monkey No. 1001, the curve of the plasma ascorbic acid remained low. Although monkeys No. 1027 and No. 1032 exhibited increases in the plasma vitamin C after being placed on one-half an orange daily, the levels fluctuated considerably. The reason for this is unknown. It is possible that the prolonged period of deficiency to which these animals were previously subjected may have rendered the mechanism controlling vitamin C labile.

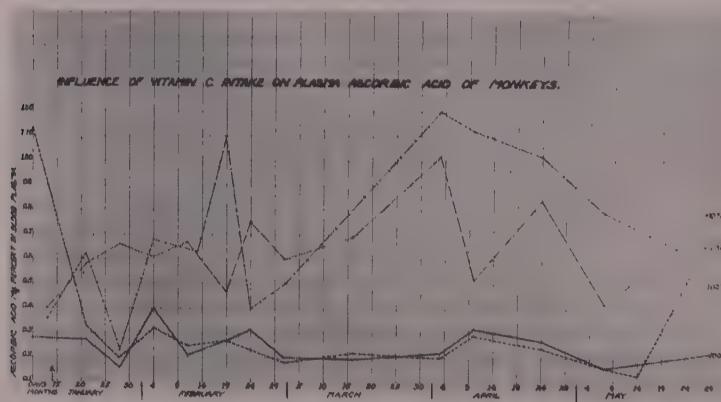


FIG. 2.

It is pertinent to note that the estimation of the reduced plasma ascorbic acid is only a measure of the immediate nutritive or metabolic level relative to vitamin C, and is dependent upon recent dietary habit to a large degree. Although it is an index of the vitamin C nutrition at the time of the test, in a single case a low level does not imply tissue injury or scurvy (either clinical or sub-clinical). The

latter results from the operation of sub-optimal or low metabolic levels over some period of time. Conversely, a good or high level would not indicate that deficiency had not operated to produce tissue injury in the past. A more accurate index of the degree of deficiency existing at the time in any given case can be had by serial determinations following administration of known vitamin C supplements. This is apparent in Table II and Fig. 2.

Summary. Experimental data is presented confirming the work of Farmer and Abt that the reduced ascorbic acid content of the blood plasma in the "normal" individual parallels the vitamin C intake. Confirmation of this is presented in a controlled dietary experiment using rhesus monkeys. For satisfactory comparative data, determinations should be made on fasting blood specimens. On the basis of the studies recorded, we believe that fasting plasma ascorbic acid levels below 0.7 mg. % are probably sub-optimal. Levels ranging between 0.7 and 0.9 mg. % would appear adequate. Optimal levels probably lie above this range. Reduced ascorbic acid plasma levels below 0.5 mg. % must be considered low.[†]

8886 C

"Three Dimension" Graphs for Correlating "Age-Weight-Gland" Relationships.

J. H. CLARK. (Introduced by L. G. Rowntree.)

From the Laboratories of the Philadelphia General Hospital.

In biometric studies of rats, it is desirable at times to compare simultaneously and graphically (1) Various organs by weight with (2) age and (3) body weight of the animal. By ordinary two dimension graphs, relationships between any 2 of these data can be well illustrated, but only by "three dimension" graphs is it possible to illustrate simultaneously the interrelationship of all 3—age, body-weight and weight of any given organ.

The construction of such graphs is simple (Chart 1): Ordinate AB forms with abscissae BC and BD an angle of 120°. AB represents age, the only constant uniform with all rats; BC body-weight and BD gland-weight. The "age-weight" is easily plotted, the line B₁C₁ paralleling BC and forming an angle of 120° (AB₁C₁). Gland-

[†] We wish to acknowledge our indebtedness to Dr. Nina Simmonds for the dietary surveys made upon the group of medical students.

weight is plotted from B_1 (corresponding to age) on line AB to D_1 (representing gland-weight), which point must be on a line perpendicular to AB projected from C_1 (corresponding to body-weight).

For normal rats all lines representing "age-body-weight" (B_1C_1) will parallel abscissa BC and form angles of 120° with ordinate AB , differing only in length. The lines representing "age-gland-weight" (B_1D_1) will vary in length and will parallel BD only when the increase in weight of the gland, with respect to age is directly proportional to the increase in body weight. If the gland is small for the age, angle D_2B_1A will be greater than 120° and line D_2B_1 short. If the gland is large, then line D_3B_1 will be longer and angle D_3B_1A

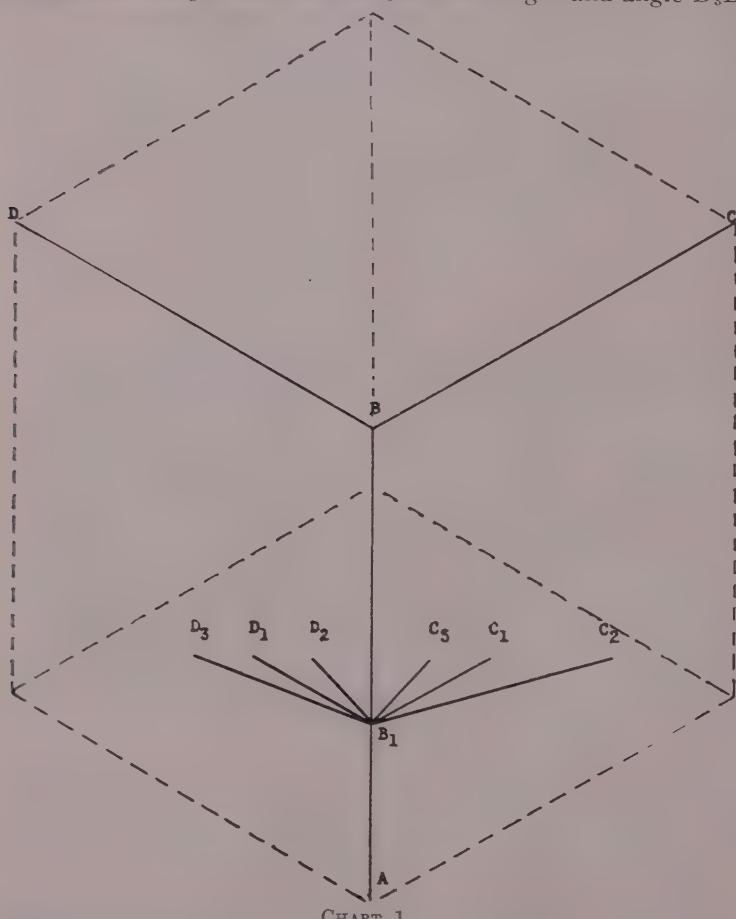


CHART 1.

Illustrates the "three dimensional" quality of the graphs. See text for construction.

more acute. If the body-weight of an animal has been increased over the normal by some experimental procedure then line C_2B_1 will be longer and angle C_2B_1A more acute; if the weight has been decreased, line C_3B_1 will be short and angle C_3B_1A more obtuse. In any case points C_2 and C_3 must always be on the line perpendicular to AB projected through C_1 (the normal body weight).

Such graphs have a "standard pattern" for any given organ at any given age-weight. In addition they have the advantage of demonstrating graphically whether the deviation from normal is in the body-weight or gland-weight.

Chart 2 shows the effect of daily injections of pregnancy urine on the ovary of immature rats for 10 and 20 days. It indicates a negligible change in body weight with a 100% increase in the weight of the ovaries of the treated rats after 20 days.

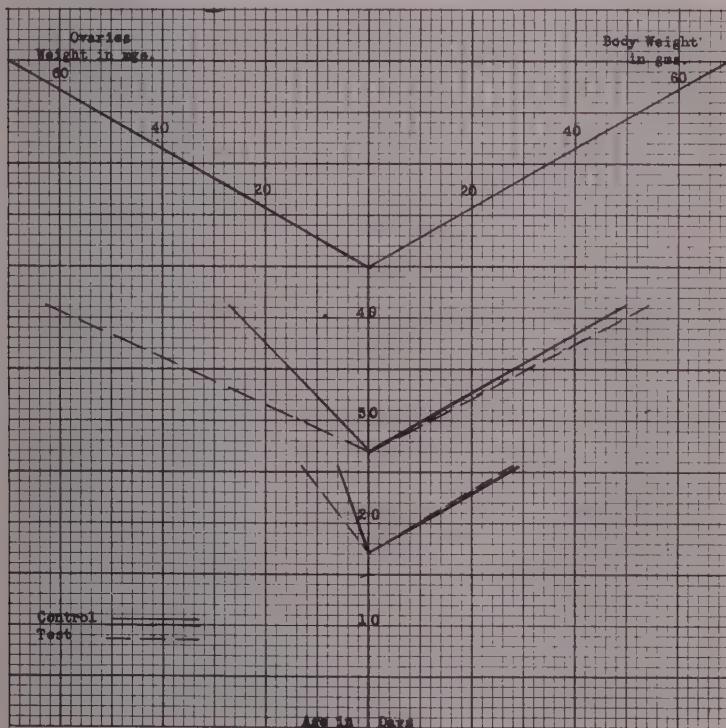


CHART 2.

Illustrates well the lack of effect on body weight of injections of pregnancy urine compared with the effect on ovarian weight. Note that the control "body-weight" graph parallels the "body-weight" abscissa, indicating that it has been the ovaries which have been disturbed experimentally.

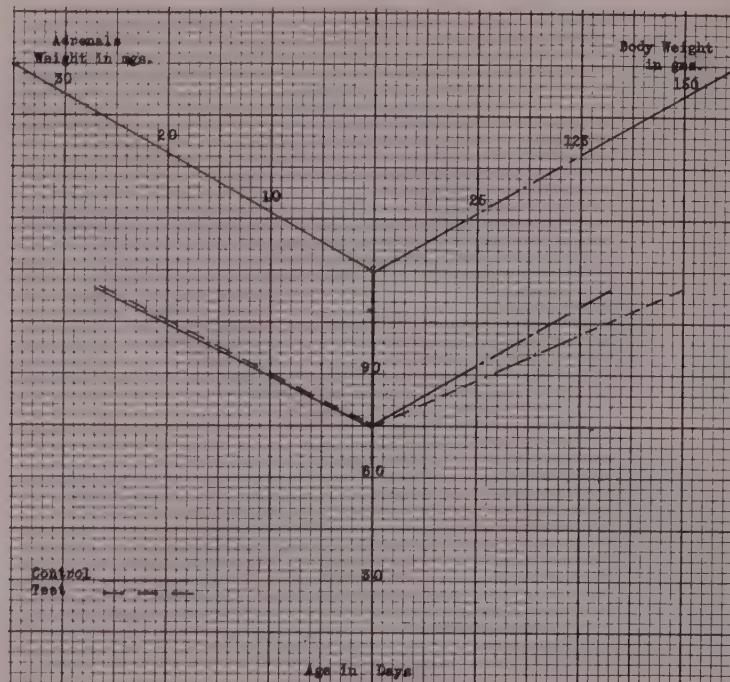


CHART 3.

Growth hormone does not disturb the weight of the adrenal but does increase body weight; ∴ graph of body weight of test rat is not parallel to the abscissa, is longer than control and the angle formed with the ordinate is more acute (see text). The break in continuity of the "body-weight" abscissa has been made to keep the chart small.

Chart 3 shows the absence of effect on the adrenal of injections of growth hormone, which has increased body weight almost 15% over the control rat.

These "three-dimension" graphs are reported with the hope that they may be of value to others interested in disturbed "age-weight-gland" relationships in demonstrating graphically the deviation from the normal in the proper direction, either body weight or gland weight.

Three Dimension Graphs for Contrasting Various Endocrine Organs of Thymus Rats with "Age-Weight-Gland" Controls.

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By ordinary two-dimension graphs, relationship between age and body weight in rats is well illustrated as well as relationship between various organs and age or body weight. Only by three dimension graphs however is it possible to illustrate the interrelation of age and body weight and also the weight of any given organ.¹ Such graphs have been particularly helpful in evaluating the influence of thymus extract on the various endocrine organs of the rat, whose general

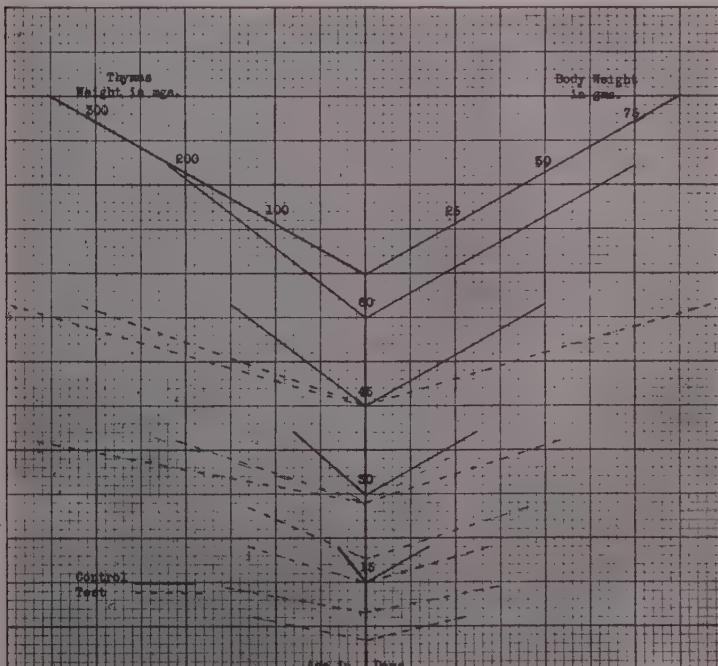


CHART 1.

Comparison of "age-body-thymus-weight" in thymus injected rats and controls. Note increase in both body-weight and thymus-weight in test strain. The larger thymus weights of rats at 15, 27 and 45 days of age are in females.

¹ Clark, J. H., PROC. SOC. EXP. BIOL. AND MED., 1936, 35, 139.

body growth is so much more rapid than normal. Thus it affords comparison with age-weight controls, in contrast to controls of comparable age but lower body weight or comparable weight and greater age. Eight thymus-injected rats exhibiting average precocity are used to illustrate the usefulness of these graphs.

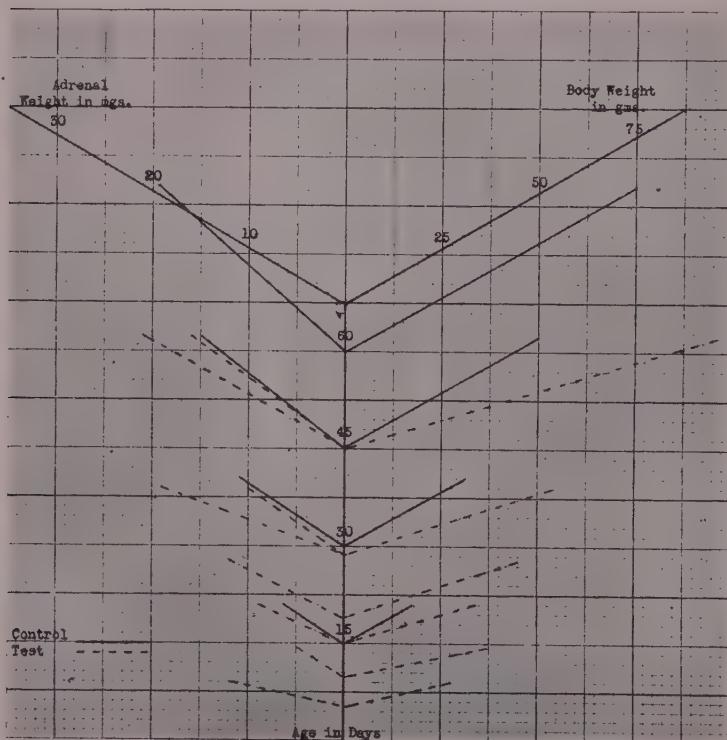


CHART 2.

Injections of thymus extract produce more marked deviation from normal in body-weight than adrenal-weight. Heavier adrenals at 15, 27 and 45 days of age are in females.

Thymus-treated rats appear grossly normal, differing mainly in their size with respect to age. Therefore the "age-weight" graph of the injected rats is not parallel to the normal (Chart 1), nor is the "age-gland-weight," since the thymus gland in thymus-treated rats is consistently heavier than normal. This increase in size is apparently due, on histologic examination, to the cortex being slightly wider than normal and less sharply demarcated from the medulla, both being crowded with lymphocytes. Hassall's cor-

puscles are less easily distinguished, seem fewer and are composed of fewer reticular cells than normal controls.

We gain the impression, through sacrificing rats and weighing the adrenals, that they tend to be below normal; plotted three dimensionally however (Chart 2) this does not seem to hold, nor was any deviation from normal observed histologically in the ordinary stains. (The graph for normal adrenals is the average between males and females for the plotted age-weight). The same observations apply to the thyroid.

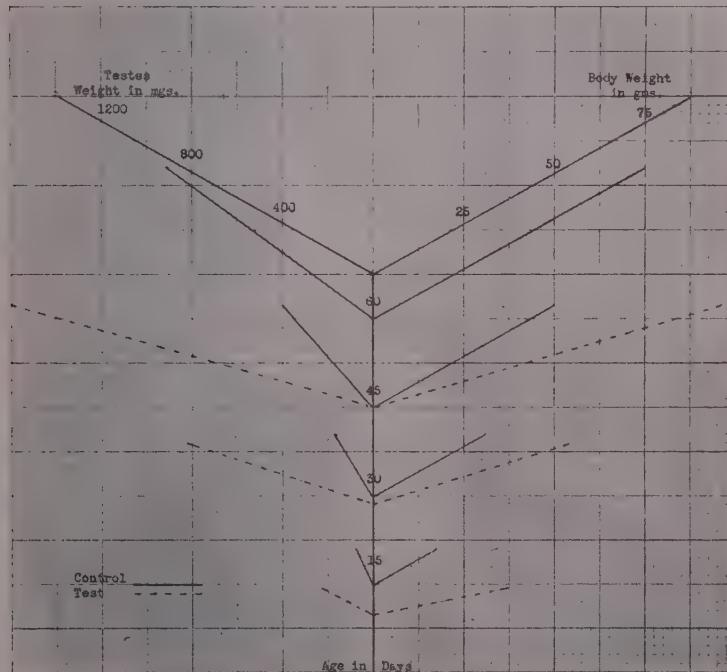


CHART 3.
Body-weight and testis-weight in thymus injected rats contrasted with normals.

The testes in treated rats descend into the scrotum at a much earlier age (2-3 days) than normal (31-40 days). They are much heavier than in control rats (Chart 3), due apparently to more rapid differentiation into the mature type of gland; lumina appear in the tubules of such testes about the 10th day and spermatids about the 25th (Fig. 1), contrasted with about the 30th and 40th days respectively in controls.

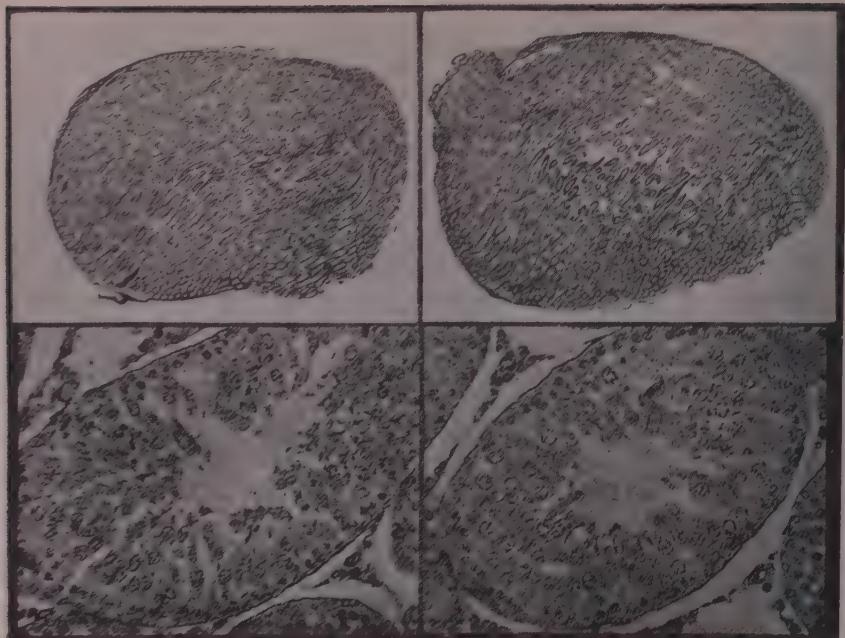


FIG. 1.

Low ($\times 11$) and high ($\times 345$) power photomicrographs of testes of 44 day old control (upper and lower left) compared with a 25-day-old thymus injected rat (upper and lower right). Testis of thymus injected rat is larger and shows more spermatids than control.

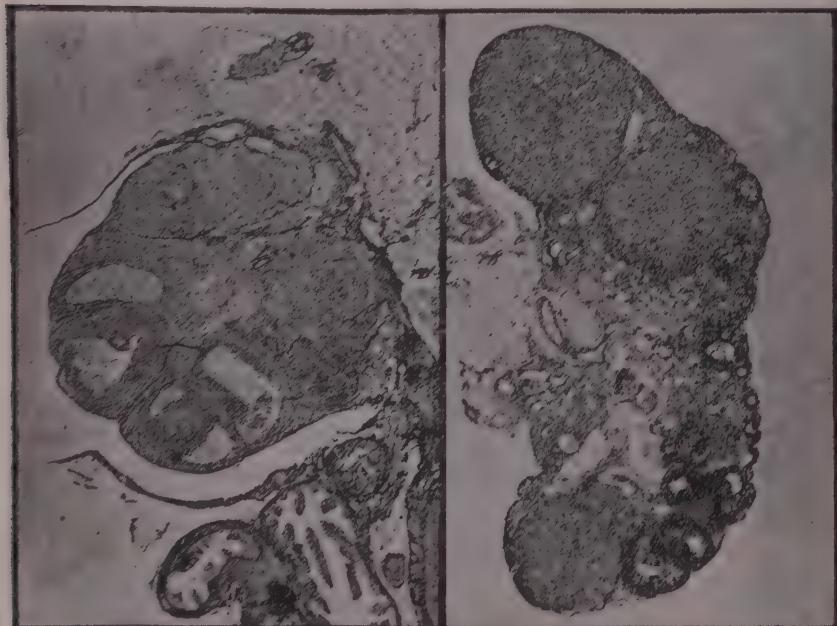


FIG. 2.

Low power ($\times 26$) photomicrograph of ovaries of a 44 day-old control rat (left) contrasted with a 9-day-old thymus injected rat (right). Note definite corpora lutea in ovary on right.

Even greater precocity in sexual development and differentiation has been noted in female rats. Our records list the opening of the vagina on the 6th day (normal 55-62 days) and corpora lutea have been observed as early as the 9th (normal 62-64 days) (Fig. 2).

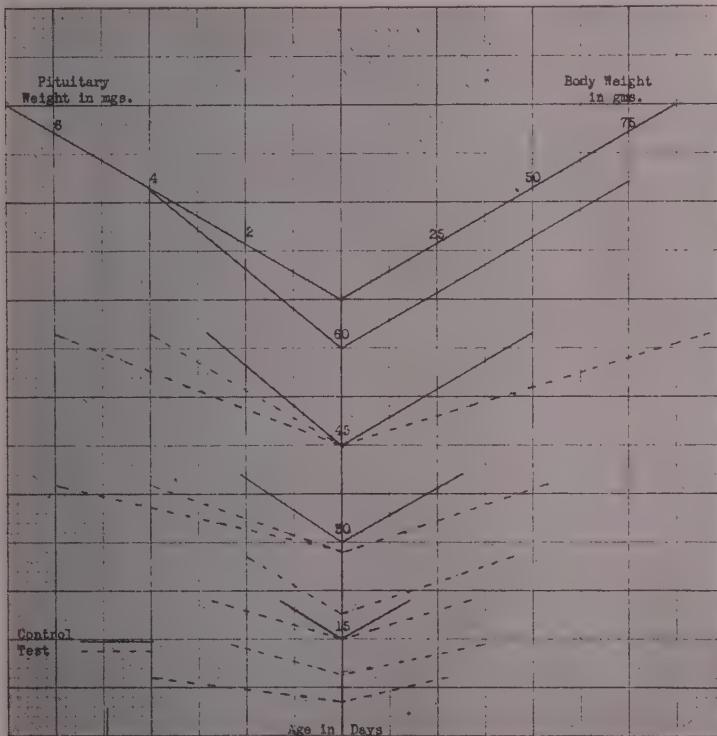


CHART 4.

Effect of thymus extract on weight of pituitary. Larger weights at 15, 27 and 45 days of age are in females.

Chart 4 shows the weight of the pituitaries of the test strain compared to those of their age-weight controls. These seem definitely in excess of normals, particularly in younger rats. In differential cell counts, the content of acidophils in the younger rats is greater than in age-weight-gland controls. This forms the subject of another report. A biometric study of certain endocrine organs of the rat is reported to demonstrate, by three-dimension graphs, the effect of thymus extract on them. This is supplemented by a brief note on the pertinent histologic deviations from normal.

Effect of Low Oxygen Tension upon Respiration and Fermentation of Isolated Cells.

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Warburg has devised the methods which make it possible to examine the respiration of isolated cells at a constantly maintained equilibrium between the cell and the gas medium and to measure quantitatively the influence of various oxygen tensions upon respiration in such a way as to eliminate diffusion as a limiting factor.¹ By these methods Warburg has determined the effect of low oxygen tensions upon the respiration of bacteria and isolated animal cells. He found the rate of oxidation of the nucleated red blood cells of geese, which he examined at 0°C., to be unchanged at oxygen pressures varying between 5 and 75 mm. Hg. (=0.7 and 10 vol. % O₂).² In *micrococcus candidans*, at 1°C. the rate of respiration even at an oxygen tension of 10⁻¹ atm. (=0.0076 mm. Hg., =0.001 vol. % O₂) was the same as in air.³ Also the respiration of non-nucleated red blood cells of rabbits poisoned by phenylhydrazin, examined at 38°C. showed no dependence upon oxygen tension.⁴ Since these investigations it has been a generally accepted axiom of physiology that cell respiration is independent of variations of oxygen tension.⁵

The only exceptions seemed to be the nitrogen-fixing bacteria and pneumococcus which were both examined with the Warburg methods under conditions of optimal gas diffusion. The relative rate of respiration of azotobacter at 2 vol. % O₂ and in air was 1:2 (Meyerhof⁶) and that of pneumococcus at 2 vol. % O₂ and in air was even 1:4.5 (Schlayer⁷).

I have examined the effect of oxygen tension upon the rate of respiration of isolated animal cells and bacteria at temperatures between 1° and 42°C. Like Warburg, I did not find any difference in the rate of respiration at oxygen tensions between 1 and 20 vol. % O₂ when working at low temperatures, nor did I find any difference

¹ Warburg, O., *Stoffwechsel der Tumoren*, Berlin, 1926.

² Warburg, O., *Erg. d. Physiol.*, 1914, **14**, 253.

³ Warburg, O., and Kubowitz, F., *Biochem. Z.*, 1929, **214**, 5.

⁴ Warburg, O., Kubowitz, F., and Christian, W., *Biochem. Z.*, 1931, **242**, 176.

⁵ Meyerhof, O., *Chemische Vorgänge in Muskeln*, Berlin, 1930, p. 9.

⁶ Meyerhof, O., and Schulz, W., *Biochem. Z.*, 1932, **250**, 35.

⁷ Schlayer, C., *J. Bact.*, 1936, **31**, 181.

at temperatures between 25° and 42° C. in old bacterial cultures, in injured nucleated blood cells, and in non-nucleated human erythrocytes. Young undamaged body cells, however, examined in their physiological environment, and bacteria in suspension media, in which they were able to grow, showed at temperatures between 25° and 42° C. a great decrease in respiration at an oxygen tension as high as 5 vol. % as compared with air (20 vol. % O₂). Under these conditions I have found a marked effect on respiration of variations of oxygen tension in *Micrococcus candidans*, *Staphylococcus aureus*, *Pseudomonas pyocyanus*, *Escherichia coli*, *Monilia albicans*, human erythroblasts and leucemic leukocytes, red blood cells of fowls and alligators and young green plant cells (pine needles). The dependence of the respiration upon oxygen tension is greatest in the youngest cells and is influenced by physical and chemical changes in the cell medium such as pH, CO₂ concentration and bicarbonate content.

There was no increase in the fermentative metabolism corresponding to the decrease of respiration. Although the rate of respiration, e. g., of geese erythrocytes, was decreased by 60% at an oxygen tension of 3.4 vol. % compared with the respiration in air, no acid formation occurred.

A typical experiment is described in the following:

Blood was taken under sterile conditions in heparin (5 mg. in 20 cc.) from the wing vein of a goose without contamination with tissue fluid. The blood was gently shaken with glass beads for 5 minutes and filtered through gauze. It was then saturated at 40° C. with a gas mixture of 2.5% CO-5% CO₂-18.75% O₂-73.75% N₂, so that the hemoglobin oxygen was almost completely displaced by CO,⁸ and pipetted into 4 Warburg manometer vessels of about 18 cc. capacity. Vessel 1 (4 cc. of blood) and vessel 2 (2 cc.) were saturated again with the same gas mixture while shaking in the thermostat. Vessel 3 (4 cc.) which contained 0.2 cc. 10% NaOH in the side bulb, in order to absorb the carbon dioxide, was saturated with 2.5% CO-18.75% O₂-78.75% N₂. Vessel 4 was saturated with 5% CO₂-95% CO in order to produce anaerobic conditions. The dry weight of cells in 4 cc. blood was 237 mg., the temperature was 40° C., the shaking speed 160-210 oscillations per minute. The readings were made every 5 minutes without stopping the manometers.

In vessels 1, 2, and 3 the metabolism was determined at 18.75% O₂ for a period of 30 minutes. Then the vessels were saturated in the thermostat with a gas mixture containing 3.4% O₂ and an equal proportion of CO to O₂ (0.45% CO-3.4% O₂-5% CO₂-91.15%

⁸ Warburg, O., *Biochem. Z.*, 1929, **214**, 4.

N_2). After another 30 minutes' observation the vessels were resaturated with the original gas mixtures of 18.75% O_2 concentration. CO_2 retention and metabolism figures were calculated according to Warburg.⁴

Table I shows that at 18.75% O_2 there is only a very small difference in the absolute respiration values between the blood cells examined at a normal pH and a physiological CO_2 tension and the cells examined at an alkaline pH in the absence of CO_2 . Decrease of oxygen tension, however, causes in the physiological medium a great decrease in respiration which is completely reversible if the cells are not kept under low oxygen pressure too long; whereas in the alkaline carbon dioxide free medium the respiration rate remains nearly unchanged.

TABLE I
Metabolism of 160 mg. Blood Cells of Anemic Goose in 1 Hour at Various Oxygen Tensions, 40° C.

| Vol. % O_2 | e.mm. oxygen consumed | | e.mm. lactic acid formed |
|---------------------|--|-----------------------------------|--------------------------|
| | in alkaline, CO_2 free medium | in unchanged physiological medium | (1 e.mm. = 0.004 mg.) |
| 18.75 | —101 | —106 | + 16 |
| 3.4 | — 96 | — 44 | + 0 |
| 0 | | | +190 |

This proves that the decrease of respiration at low oxygen tensions is not due to insufficient diffusion of oxygen from the gas space or the suspension medium into the cells, since all the conditions which might influence the diffusion (cell volume, oxygen tension, shaking speed) are identical throughout.

The fact that cellular respiration in an alkaline, CO_2 -free medium as well as at low temperatures is largely independent of oxygen tension, is probably the chief reason why previous workers did not find the marked effect of oxygen tension upon respiration. Even the dissociation of oxyhemoglobin at decreasing oxygen tension might have been overlooked if the dissociation had only been measured at low temperatures and in alkaline CO_2 -free media. For each of these three factors affects the affinity of hemoglobin for oxygen to such an extent that one must go down to very low oxygen tensions before the dissociation of oxyhemoglobin begins.

In complete absence of oxygen a great amount of lactic acid is formed while at an oxygen concentration of 3.4 vol. % in spite of 60% decrease in respiration no lactic acid appears. No relationship was found between the decrease in respiration and the occurrence of glycolysis. The Pasteur reaction, the disappearance of the anaero-

bic splitting metabolism under aerobic conditions, was not dependent upon the rate of respiration but upon the concentration of oxygen.

8889 P

Effect of Crystalline Vitamin C (Ascorbic Acid) on Tolerance to Tuberculin.

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Columbia University.*

One of the characteristics of the reaction of tuberculous animals to a large dose of tuberculin is congestion and capillary hemorrhage in all tissues, particularly in those containing tubercles. This is attributed to altered cell permeability and capillary dilatation. Since vitamin C has been shown to decrease capillary fragility (Dallendorf¹), and has been employed with encouraging therapeutic results in certain types of hemorrhage (Willstaedt²), we were led to study this substance in relation to tuberculin intoxication. It is of interest to note that about 10 years before the discovery of the anti infectious and antitoxic action of vitamin C (Harde,³ Jungeblut and Zwemer,⁴ King and Menten,⁵ Jungeblut⁶), Bieling⁷ had already found that a dose of tuberculin which killed only one of 2 tuberculous guinea pigs maintained on a normal diet, sufficed to kill 2 scorbutic tuberculous guinea pigs.

The effect of crystalline vitamin C (Ascorbic acid*) on tuberculin was investigated in tuberculous guinea pigs fed a normal diet which included an abundance of fresh lettuce as a natural source of vitamin C. Ascorbic acid mixed *in vitro* with skin test doses of tuberculin prior to intracutaneous inoculation in tuberculous guinea pigs failed to inactivate the tuberculin in tests on 12 animals. Nor did prolonged pretreatment of 7 tuberculous guinea pigs with ascorbic acid reduce the reactivity of the skin to small doses of

¹ Dallendorf, G., *J. Am. Med. Assn.*, 1935, **104**, 1701.

² Willstaedt, H., *Klin. Woch.*, 1935, **14**, 1705.

³ Harde, E., *Compt. rend. Acad. Sci.*, 1934, **199**, 618.

⁴ Jungeblut, C. W., and Zwemer, R. L., *Proc. Soc. Exp. BIOL. AND MED.*, 1935,

32, 1229.

⁵ King, C. G., and Menten, M. L., *J. Nutrit.*, 1935, **10**, 129.

⁶ Jungeblut, C. W., *J. Exp. Med.*, 1935, **62**, 617.

⁷ Bieling, R., *Zeit. f. Hyg.*, 1925, **104**, 518.

* We are indebted to Merck & Co. for a generous supply of crystalline vitamin C (Cebione; Cevitamic Acid Merck).

tuberculin. We were also unable to determine any ability of the vitamin to inactivate tuberculin either *in vitro* or *in vivo* when the tuberculin was used in a dose large enough to cause death of control tuberculous guinea pigs. The *in vitro* test was performed by injecting 6 tuberculous guinea pigs with tuberculin previously incubated with vitamin C; 6 control tuberculous animals were injected with tuberculin alone. Four animals died of tuberculin shock in each group. Two experiments were performed on the effect of injecting ascorbic acid intravenously in doses of from 0.5 to 75 mg. into tuberculous guinea pigs just prior to inoculation with a large dose of tuberculin, lethal for control tuberculous guinea pigs. In both experiments all 12 animals receiving ascorbic acid followed by 250 or 500 mg. of tuberculin died of typical tuberculin shock. Another experiment was tried in which 4 tuberculous guinea pigs were injected subcutaneously with 5 mg. of ascorbic acid daily for one month. These guinea pigs, together with 4 non-treated tuberculous controls, were then injected subcutaneously with 450 mg. of tuberculin. Only 2 of the control guinea pigs died, whereas all 4 ascorbic acid treated animals died. Thus, in none of these experiments with lethal doses of tuberculin had any tuberculin-inactivating power of ascorbic acid been demonstrated. When, however, the tuberculin was injected in somewhat smaller but repeated doses, large enough in the aggregate to be fatal for control tuberculous animals, it was found that daily administration of ascorbic acid usually resulted in survival of the animal.

This observation was first made in a preliminary experiment on 6 guinea pigs infected subcutaneously with 0.001 mg. of bovine tubercle bacilli. Two months after infection the animals were divided into 2 groups. Three guinea pigs were injected subcutaneously with 5 mg. of ascorbic acid daily throughout the course of the experiment; the other 3 guinea pigs served as controls. During this same time both groups received injections of Old Tuberculin[†] twice weekly according to the following schedule: Four 100 mg. doses intracutaneously, then subcutaneous injections of 200 mg., 200 mg., 225 mg., 250 mg., 250 mg., and 250 mg. One control guinea pig died after the first subcutaneous injection of tuberculin, another died after the fifth, and the third control animal died after the sixth subcutaneous injection. All 3 vitamin C treated animals survived the entire series of tuberculin injections and, furthermore, proved able to tolerate several additional 250 mg. doses of tuberculin.

[†] Koch's Old Tuberculin was obtained through the courtesy of the Bureau of Laboratories, Department of Health, New York City.

The increased tuberculin tolerance of vitamin C treated animals was again convincingly demonstrated in a subsequent experiment. Sixteen male albino guinea pigs were infected subcutaneously with 0.001 mg. of a bovine strain of tubercle bacillus (B1). Eighteen days later, the guinea pigs were grouped in pairs on a basis of comparable weight, one animal of each pair receiving subcutaneous injection of 5 mg. of ascorbic acid every day, and the other animal serving as control. The ascorbic acid was dissolved in sterile distilled water just prior to injection. One guinea pig had died of intercurrent infection 5 days after inoculation with tubercle bacilli, leaving an unmated animal in the ascorbic acid treated group. All guinea pigs received 2 injections of Old Tuberculin weekly, starting 23 days after infection. The schedule of tuberculin doses (in milligrams) was as follows: (1) 50, (2) 100, (3) 150, (4) 200, (5) 250, (6) 300, (7) 400, (8) 550, (9) 700, (10) 700, (11) 900, (12) 900, (13) 1100, (14) 1100, (15) 1300, (16) 1300, (17) 1600, (18) 1600 mg. The first dose of tuberculin was given intracutaneously, all others subcutaneously. Whenever an animal died, its mate in the other group was killed for purposes of comparing the extent of the disease and the vitamin C content of the adrenal.

The first death occurred in a control animal following the ninth injection of tuberculin. At the 15th injection, 4 additional deaths had occurred, all of which were in the control group. At the 17th injection the first of the vitamin C treated animals died. This animal showed a non-tuberculous peritonitis at autopsy. At the 18th injection, the last control animal died. Thus, there was only one death in the vitamin-injected group as compared to 6 deaths in the control group. The unmated guinea pig which had been included in the vitamin C treated group had also survived the entire series of tuberculin injections. This animal proved able to survive additional tuberculin injections of 1600, 2000, and 2500 mg. It was apparent, therefore, that the animals receiving ascorbic acid daily were better able to tolerate repeated large doses of tuberculin.

The adrenal glands of the animals in this experiment were roughly estimated for vitamin C content by treating the sliced organ with 0.4% silver nitrate for one-half hour, and examining for visible blackening in the cortex. All the ascorbic acid treated animals showed the presence of reducing substances at autopsy, with the exception of the one animal which had succumbed spontaneously. On the other hand, of the 6 control guinea pigs which had died of tuberculin shock, 5 failed to reveal any trace of reducing substance in the adrenal, and the sixth gave a much weaker reaction than did the paired animal of the treated group. The one control animal

which did not succumb to tuberculin showed an appreciable degree of blackening in the adrenal. These observations suggested that tuberculin death was associated with depletion of vitamin C, whereas those animals that survived tuberculin shock always showed vitamin C in the adrenal.

Details of the autopsy findings will be reported elsewhere. We note here that in 6 of the 7 pairs of autopsies, the control animal showed more extensive tuberculosis than did the corresponding ascorbic acid treated animal. The precise mechanism for the observed effect of vitamin C on tuberculosis and tuberculin tolerance remains to be clarified. No evidence was obtained for a direct inactivation of tuberculin by vitamin C. Nevertheless, the results suggest that vitamin C may prove of value in tuberculosis by combatting the prolonged toxemia of the disease. We are at present testing this hypothesis in both clinical and animal experiments.

Summary. Daily injection of crystalline vitamin C increased the tolerance of tuberculous guinea pigs to repeated large doses of tuberculin.

8890 C

Effect of Ether Anesthesia and Amytal Anesthesia on the Erythrocytic Findings in Control and Splenectomized Dogs.

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FRANK C. MANN.

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Minn.*

In a study of the influence of ether anesthesia and amytales anesthesia on the blood of the dog it was shown by Searles and Essex¹ that following ether anesthesia there was usually a marked increase in the erythrocyte count, the value for the hemoglobin and the hematocrit determinations. When amytales was used as the anesthetic there was usually a decided decrease in the erythrocyte count, the value for the hemoglobin and the hematocrit readings when compared with these findings before the induction of anesthesia. Since it is a common observation that the spleen of the dog is usually markedly dilated following amytales anesthesia, the decrease in the

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¹ Searles, P. W., and Essex, H. E., *Proc. Staff Meet. Mayo Clinic*, 1936, **11**, 481.

erythrocyte count, the value for the hemoglobin and the hematocrit values led to the conclusion that amyntal anesthesia causes a sequestration of the erythrocytes in the spleen. In a small series of experiments the evidence was highly suggestive that such a phenomenon occurred since splenectomized dogs failed to show a marked change in the erythrocytes, the concentration of hemoglobin and the hematocrit values after amyntal had been given.

Because of the fundamental nature of these observations it was thought advisable to study a larger series of animals from this point of view. Since the personal equation may be of considerable importance in an investigation involving a quantitative study of erythrocytes, we took particular care to eliminate such influences in these experiments. Sixteen dogs were selected and submitted to us for a critical study of the part played by the spleen in altering the number of erythrocytes in the peripheral blood when ether or sodium amyntal was used as an anesthetic. The condition of the animals as far as the spleen was concerned was unknown to us until the study was completed. Some of the animals possessed spleens while others had been without spleens for varying lengths of time. If the spleen is largely responsible for the increase of erythrocytes in the peripheral blood following administration of ether and for the reduction in the number of circulating erythrocytes following amyntal anesthesia, it should be possible to determine the normal and splenectomized dogs in the series by observing the changes in the erythrocyte count, the concentration of hemoglobin and alterations in the hematocrit values. To settle this question the experiments reported here were done.

In order to study the reaction of the animals to the 2 anesthetics, the animals were first studied while they were under ether anesthesia and a week later when amyntal was used as the anesthetic.

In obtaining the control samples of the blood, care was taken to insure a minimum of excitement on the part of the animal during the process. The blood was withdrawn from the right or left saphenous vein. In the experiments with ether, immediately after the control samples of blood were taken, the animals were anesthetized in an ether chamber, after which surgical anesthesia was maintained for 30 minutes by the auto-inhalation method. The second sample of blood was taken at the end of a 30-minute period of anesthesia and the administration of ether was discontinued. An hour after the administration of ether was discontinued, a third sample of blood was taken.

When amyntal was used, 50 mg. for each kilogram of body weight was injected into the saphenous vein immediately after removal of

the control sample of blood. The second and third samples of blood were removed 30 and 60 minutes later respectively.

In order to insure uniformity, the samples of blood for the 3 determinations were taken by the same individuals. All the data on the erythrocyte count were assembled by one group, the data on the value for the hemoglobin and the hematocrit readings were assembled by another group. The Sheard-Sanford hemoglobinometer was used for determining the values for the hemoglobin, and the hematocrit studies were made by the use of standard hematocrit tubes. For the hematocrit readings the blood was withdrawn into a solution of heparin and the tubes were centrifuged for 30 minutes at approximately 1500 revolutions per minute.

An analysis of the data on the series of 16 dogs indicated that 7 were normal and 6 had been splenectomized; one was listed as indefinite but probably normal. The remaining 2 were listed as indefinite and the studies on these dogs should be repeated. When our findings were checked with the histories of the animals used it was interesting to note that all the splenectomized dogs in the series had been so designated and all the normal dogs on which we had committed ourselves, had normal spleens. According to the records, the 3 indefinite animals each possessed a spleen.

The results with ether anesthesia were much less reliable for determining the presence or absence of the spleen than were the results with amytal anesthesia. This is not surprising since the state of the spleen at the time the control samples of blood were taken would probably vary considerably. If the spleen, as a result of excitement or other causes, was in a very contracted state, the effect of ether on the erythrocyte count and the concentration of hemoglobin and the hematocrit readings would be much less marked than it would if the spleen were widely dilated. That is, an already partially contracted spleen could not pour into the circulating blood as many erythrocytes in response to ether anesthesia as could a fully dilated spleen.

Conclusions. Ether anesthesia causes profound constriction of the spleen and usually produces a marked increase in the erythrocyte count, the value for the hemoglobin and the hematocrit value while sodium amytal causes the spleen to dilate markedly, which removes a considerable percentage of erythrocytes from the circulation. Our results are in full agreement with those of Searles and Essex.

8891 P

Coagulation of Blood by Proteolytic Enzymes (Trypsin, Papain).

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Crude or crystalline trypsin in proper concentration coagulates human, dog, rabbit, guinea pig and horse plasma or whole blood. It does not clot fibrinogen directly, but reacts with prothrombin to form thrombin. This activation is independent of the presence of either calcium or platelets. It follows that neither of these is necessarily an intrinsic part of thrombin. Trypsin also coagulates blood *in vivo*, which suggests that circulating blood contains free and reactive prothrombin, rather than an inactive hypothetical complex from which prothrombin is liberated only after blood is shed.

An excess of trypsin digests fibrinogen, prothrombin and thrombin; and too little trypsin has no effect. The activation of prothrombin therefore takes place within a relatively narrow zone of enzyme concentration, which varies directly with the amount of protein present. At this optimum zone, there is a dynamic equilibrium between the activation of prothrombin to thrombin and the destruction of both reagents by the enzyme.

Since trypsin, in activating prothrombin, has qualitatively the same effect as the physiological system calcium plus platelets (or calcium plus tissue extracts), it is tentatively suggested that these systems contain a proteolytic enzyme similar to trypsin which reacts with prothrombin to form thrombin.

It has been further found that the proteolytic enzyme papain, freed of calcium, nevertheless coagulates whole blood, plasma, or fibrinogen. In this case, the enzyme does not activate prothrombin, but acts directly on fibrinogen to form a soft fibrillar gel resembling fibrin, with a marked tendency to resolution. If one admits this clot to be fibrin, this constitutes strong evidence that the physiological coagulant thrombin is also a proteolytic enzyme with a specific reactivity for fibrinogen.

The working hypothesis here suggested as to the mechanism of physiological coagulation is, therefore, that the calcium and platelet together contain a proteolytic enzyme which, like trypsin, transforms prothrombin to thrombin; and that the product of this reaction, thrombin, is itself a proteolytic enzyme which, like papain, converts fibrinogen to fibrin. A complete analogy for this hypoth-

esis is found in the recently reported activation of chymotrypsinogen by trypsin to form a new proteolytic enzyme, chymotrypsin (Kunitz and Northrop).¹

8892 C

Effect of Cinchophen on the Liver and Other Tissues of the Dog.*

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During a study of peptic ulcers produced by the administration of cinchophen we had the opportunity of studying the effects of large amounts of cinchophen on the liver and other organs of the dog. In view of the divergence of opinions regarding the effects of cinchophen on the livers of experimental animals, it seems advisable to record our observations on this subject.

Specimens of liver were obtained from 131 dogs which were normal in every case before the experiment was begun. They were given the routine kennel care and were fed a balanced diet of a calculated weighed amount sufficient for their caloric requirements. Cinchophen was for the most part administered orally; in a few cases it was given rectally, parenterally, or through intestinal fistulas.

Thirty-one dogs, whose average weight was 17 kg., were daily given 2 gm. of cinchophen well mixed with their food for a period varying from 3 to 60 days. The average length of time over which administration of the drug to this group was continued was 27 days; the average total dose of cinchophen was 50 gm.

Fifty-four dogs were given 2 gm. of cinchophen daily, with an occasional rest day, for an average of 30 days but in a few cases over a period of time as long as 114 days. To this group the amount of cinchophen administered varied from 36 to 228 gm. during the course of treatment.

Thirteen dogs, whose average weight was 13 kg., were given varying doses of cinchophen by routes other than by mouth, the usual dose exceeding 1 gm. daily. To members of this group an average of 21 gm. of the drug was given in an average of 13 days.

¹ Kunitz, M., and Northrop, J. H., *J. Gen. Physiol.*, 1935, **18**, 433.

* Submitted for publication September 23, 1936.

Twenty dogs, whose average weight was 8 kg., were given 1 gm. of cinchophen mixed with their food 5 days of each week; no cinchophen was given on the last 2 days of each week. The smallest total dose given to a member of this group was 22 gm. in 30 days; the largest total dose was 423 gm. in 630 days. The average for the group was 198 gm. given in an average of 270 days.

The 13 dogs remaining were fed the same diet but were not given cinchophen. These dogs were killed at varying, comparable intervals and were used as controls.

The majority of the animals were killed at the end of the period of administration of cinchophen. Some were explored surgically at this time and the gross appearance of the organs was noted; a recovery period of variable time was allowed and these dogs were then killed. All dogs killed were given light ether anesthesia and were then bled from the femoral arteries.

At the time of necropsy a record was made of the gross appearance of all the organs. Multiple sections of the heart, lungs, liver, pancreas, spleen, kidneys, adrenal glands, and occasionally the gall-bladder, were fixed for histologic study.

Gastric lesions developed in all but 2 of the dogs which received cinchophen. The greatest changes in the other tissues were always noted during the first 10 days of administration of cinchophen. During this time a toxic condition usually developed, followed by a gastro-intestinal disturbance associated with varying degrees of nausea, vomiting, tarry diarrhea, and in some cases, anorexia. Since these symptoms were all manifestations of the development of peptic ulcer, we shall not discuss them further. The toxemia produced in the first few days usually subsided and the dogs then tolerated the drug fairly well. The toxic manifestations increased in a few cases, however, but these dogs usually died.

A slight yellowish color of the entire liver and kidneys was noted in a few of the animals killed during the initial stage of toxicity. A variable degree of vacuolization of the hepatic cells and of the tubular epithelium in the kidneys was seen microscopically. A few dogs had similar fatty changes in the cardiac muscle, and at times some cloudy swelling was seen in the liver. No gross or microscopic changes were noted in the other organs.

These mild pathologic changes varied with the degree of toxicity, and the toxemia varied with the dose of cinchophen. If the animal was markedly toxic, and this was true in only 3 or 4 cases, fatty degeneration was marked; if, on the other hand, the toxicity was no greater than that ordinarily produced by peptic ulcer, the fatty

change was slight, although at the same time it was more than that seen in normal dogs.

Little change was noted in any of the organs at the end of from 30 to 60 days, and the dogs killed after the administration of cinchophen for from 100 to 630 days had normal-appearing organs both grossly and microscopically. At no time was jaundice seen, and at no time was cirrhosis produced or the structural framework of the liver altered. In the kidneys of dogs fed cinchophen there was no more evidenced nephritis than in the kidneys of control animals, although during the acute toxic stage there was slightly more tubular degeneration in the kidneys of these dogs than was seen in the kidneys of the control animals. Pancreatitis was not noted at any time, and the gallbladder, adrenal glands, lungs, and spleen were consistently normal.

8893 P

Blood Electrolyte Studies in Experimental Acute Liver Injury Produced by Arsphenamine in Dogs.*

LOUIS J. SOFFER. (Introduced by W. T. Longcope.)

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In the present investigation we have attempted to determine changes in the blood electrolytes following the production of hepatic parenchymal damage with arsphenamine. The studies included determinations of the blood non-protein-nitrogen, urea, sugar, CO₂ content, sodium, chloride, potassium, calcium, phosphorus, magnesium, total proteins, and lactic acid, as well as complete hematologic studies.

Liver damage was produced in 6 dogs by injection of arsphenamine intravenously. Animals were kept on a diet of raw meat to which was added 2 gm. of salt daily for 2 weeks before the experiment was started. Arterial blood for determination of various electrolytes was collected anaerobically before injection of arsphenamine. Forty to 80 mg. per kilo of body weight of freshly prepared arsphenamine was then injected intravenously. About 30 minutes later this was followed by vomiting which lasted for a few minutes before subsiding entirely. In 2 animals, a single injection

* This work was aided by a grant from Merck and Company.

of the drug was adequate to produce icterus. Four of the 6 animals required subsequent injections of arsphenamine to produce jaundice. Death followed in all animals about 20 to 24 hours after the last injection of the drug. Arterial blood samples for electrolytes were again collected when the animal appeared definitely icteric. Six to 8 hours after appearance of jaundice the dogs became comatose and died. At no time were there convulsive seizures.

Upon death a necropsy was performed and sections of liver, kidneys, and intestines were removed for microscopic study. In 2 instances considerable congestion of the small intestine and rectum was found. The kidneys appeared grossly normal in all instances. The livers felt firm, but had a distinctly mottled appearance. The lobules were well outlined and were surrounded by punctate hemorrhages. The remaining organs appeared grossly normal. Microscopically the liver showed the most pronounced changes. Extensive central necrosis was present in all instances. The degree of the destructive process varied in the animals and it was estimated that from one-third to well over three-quarters of the individual liver lobules was destroyed. There were some well preserved cells around the periportal spaces, but even here some necrosis was present. In many areas the necrosis extended to the portal vein. The cellular structure of the lobule was entirely destroyed. The kidneys showed much less marked changes. Many of the glomerular capsules showed the presence of coagulated fluid. There was no actual damage, however, to the glomeruli. There was occasional necrosis of some of the cells of the tubular epithelium.

Results. Five of the 6 dogs developed icterus. The blood bilirubin varied from 2.0 to 16.0 mg. %, while the qualitative Van den Bergh reaction varied from a delayed biphasic to a direct reaction. The one animal that failed to develop icterus did have considerable hepatic parenchymal destruction, which was less, however, than was present in the other animals. The blood electrolyte pattern in this dog showed the characteristic alterations to be described, although to a lesser extent than in the animals in which the liver was more extensively damaged.

In all cases there occurred a marked hemoconcentration as determined by hematocrit studies. The reduction of plasma volume varied from 13.8 to 32.0%. This was associated with a proportionate increase in the number of red blood cells, but with no changes in the intrinsic character of these cells. Mean corpuscular volume, mean corpuscular hemoglobin, and the hemoglobin concentration remained unaltered throughout the experiments.

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TABLE I.
Serum Electrolyte Studies in Acute Hepatic Parenchymal Damage.

| | Dog No. | N.P.N. mg. % | Urea mg. % | CO ₂ Content m.eq./l | K m.eq./l | Ca mg. % | Pb mg. % | Mg mg. % | Lactic Acid mg. % | Total Proteins gm. % | Quail, Van den Berghe | Quart, Van den Berghe | Leiterus Index (units) | Plasma Vol. | mg./Karsphenamin intrav. | | | | | | |
|---|---------|--------------|------------|---------------------------------|-----------|----------|----------|----------|-------------------|----------------------|-----------------------|-----------------------|------------------------|-------------|-----------------------------|-----------|---------|--------------|--------------|------------|-------|
| 1 | 26 | 11 | 80 | 24.4 | 144.6 | 114.4 | 4.0 | 9.6 | 3.4 | — | 16.4 | 5.6 | neg. | 0.5 | 6 | 50.5 | 11/26 | - 2:30 P.M. | Control 60 | 76cc. | |
| | 84 | 53 | 16 | 12.5 | 145.8 | 107.0 | 13.5 | 9.5 | 12.9 | — | 85.0 | 5.4 | delayed | 2.5 | 20 | 34.5 | 11/27 | - 12:10 , | Dog died | | |
| 4 | 36 | 18 | 85 | 29.5 | 145.3 | 107.5 | 4.1 | 11.8 | 6.6 | 1.6 | 19.5 | 5.5 | Biphasic | 0.5 | 6 | 56.5 | 12/7 | - 10:30 A.M. | Control 53 | 78cc. | |
| | 35 | — | 85 | — | — | — | — | — | — | — | neg. | neg. | neg. | 12/13 | — | 2:45 P.M. | -60 | 88cc. | | | |
| | 88 | 41 | 20 | 19.7 | 138.0 | 100.4 | 11.3 | 11.6 | 18.0 | 4.3 | 80.0 | 5.1 | delayed | 3.6 | 56 | 48.1 | 12/18 | - 8:00 , | 101.5cc. | | |
| | 8 | 31 | — | 83 | 26.4 | 147.0 | 110.6 | — | 10.4 | 3.5 | — | 13.4 | 4.9 | Biphasic | 0.5 | 6 | — | 7/8/35 | - 10:00 A.M. | Control 60 | 56cc. |
| | 108 | — | 100 | 19.5 | 146.9 | 93.6 | — | — | 6.6 | — | 35.8 | 5.2 | Direct | 10.0 | 100 + | — | 7/10 | - 10:00 , | -70 | 66cc. | |
| | 10 | 30 | 17 | 70 | 26.7 | 143.3 | 111.4 | 4.9 | 10.0 | 4.4 | 1.8 | 11.7 | 4.8 | neg. | 0.5 | 6 | — | 7/11 | - 3:30 P.M. | -80 | 75cc. |
| | 76 | 56 | 32 | 17.2 | 138.4 | 101.6 | 5.6 | 9.5 | 15.4 | 2.4 | 47.0 | 4.8 | Prompt | 3.7 | 100 | 56.4 | 7/12 | - 8:00 , | Dog died | | |
| | 12 | 30 | 12 | 88 | 22.4 | 140.3 | 109.0 | 4.1 | 10.2 | 4.0 | 1.2 | 13.4 | 7.0 | Biphasic | 0.5 | 6.0 | 66.1 | 7/19/35 | - 10:00 A.M. | Control 50 | 61cc. |
| | 128 | 100 | 92 | 14.6 | 138.2 | 94.8 | 5.7 | 9.3 | 16.0 | 2.4 | 24.6 | 6.5 | very faint delayed | 0.5 | 7.0 | 56.4 | 7/20/35 | - 10:30 , | -50 | 61cc. | |
| | 14 | 34 | 18 | 72 | 27.6 | 143.0 | 104 | 5.9 | 10.2 | 3.3 | — | 10.4 | 6.1 | Biphasic | 0.5 | 5.0 | 62.1 | 7/29/35 | - 10:00 A.M. | Control 40 | 45cc. |
| | 40 | 25 | 60 | 90 | 23.4 | 139.0 | 99.4 | — | 9.8 | 3.8 | — | 29.1 | 5.6 | Direct | 5.7 | 60 | 57.2 | 7/30/35 | - 11:00 , | | |
| | 90 | 75 | 50 | 21.4 | 137.1 | 83.8 | 7.6 | 10.4 | 11.4 | — | 70.0 | 6.1 | Direct | 16.0 | 100.0 | 52.4 | 7/31/35 | - 11:15 , | Dog died | | |

Alterations in the blood electrolytes at the height of the jaundice were marked. All animals developed acidosis, reduction in the CO₂ content of the blood varying from 22 to 50%. The acidosis was due essentially to a considerable increase in lactic acid in the blood. This increase varied from twice to 7 times the control values. The increase in lactic acid was roughly proportional to the extent of liver damage. All the dogs showed a considerable drop in serum chlorides. This drop varied from 7.2 to 20.2 milli-equivalents per liter. No change occurred in the serum sodium values. The inorganic phosphorus of the serum increased from 1½ to almost 4 times the original control values, while the calcium remained remarkably constant. In 2 animals there occurred an increase in serum potassium values, and in the 3 animals in which magnesium studies were made the original control figures were doubled or trebled. An elevation of non-protein nitrogen and urea occurred in all cases. Four of the animals developed a definite hypoglycemia, the blood sugars being 16, 20, 32, and 50 mg. %. The development of hypoglycemia may be explained by the fact that following extensive injury to the liver this organ is incapable of converting lactic acid into glycogen. As a result of this incapacity there occurs an accumulation of lactic acid in the blood, and eventually a depletion of liver glycogen which is the major available source for blood sugar.

8894 C

Effect of Certain Drugs on After-Contraction.

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When voluntary muscles are put under tension against resistance through conscious effort, and then allowed to relax with the resistance removed, there follows an involuntary contraction in the original direction. Thus, as has long been known, if one stands against a wall, pushing the hand strongly against it, with the arm held stiff, on stepping away from the wall, the arm slowly rises toward a horizontal position. This phenomenon, which may be called after-contraction, has been studied by Kohnstamm, Csiky and others, who have given various explanations of its mechanism, none of which, however, seems adequate. In view of the more recent advances in the physiology of the nervous system, it seemed to us that further

study of the after-contraction would be of value. The present report deals with some aspects of the reaction.

The after-contraction may be obtained from any voluntary muscle group, and in these experiments the movement studied was flexion at the hip joint, the leg being extended. The subject stood on a platform, supporting his weight on one foot. To the other foot, hanging free, was attached a cord, passing over a pulley and carrying a fixed weight. Also attached to this foot was a string connected with a system of levers arranged to record the leg movement on a kymograph. At a given signal, the subject lifted the weight off its support by raising the extremity, and held it in a fixed position for a definite period. Then, at a second signal, the leg was relaxed and the weight disconnected. After a variable latent period the leg slowly rises, without conscious effort on the part of the subject and, indeed, somewhat to his astonishment.

Sixty or more subjects have been studied. In the great majority of instances the reaction occurred as expected. In a few, however, it was obtained only after some practice. Six kilos was the weight usually employed, and 15 seconds the time of supporting it. Variations in either factor altered the character of the after-contraction.

Using the method described, changes in the response of normal individuals under varying conditions, and in patients with neurological disorders have been studied, and will be described at a future time. In this communication the effects of some of the drugs studied will be reported. These are bromides, chloral, barbital, commonly used depressants, and caffeine and strychnine, stimulants.

Of 10 subjects given bromides (2 G) all showed a marked reduction of the after-contraction, and in 7 it was absent. The effect came on in 30 minutes or more and persisted for several hours. In spite of this marked effect, subjective symptoms were absent or mild. **No change in the knee jerk was observed.**

Eight subjects were given caffeine in dosage from 0.15 to 0.5 G. In 5 of these there was no appreciable change in the after-contraction. In 2 there was a moderate increase and in one a marked increase (500%). In contrast to this lack of uniformity of caffeine action on normal subjects, caffeine in small doses, corresponding to that in a cup of coffee, completely offset the depression caused by bromides. The antagonism was only temporary, the bromide effects returning in an hour or more.

Barbital was given to 6 subjects in relatively large doses, 0.3-0.6 G. In 3 instances there was no effect, in 2 a slight decrease, and in one no after-contraction. Two of these subjects were given bromide at one time and barbital at another. In these, the bromides abolished the after-contraction with no subjective symptoms, the

barbital was without effect on the after-contraction although marked mental depression was experienced.

Seven subjects were given chloral hydrate (0.6 G). In 3 there was no effect on the after-contraction, in 2 some lessening and in 2 an appreciable increase. Most of these experienced mental depression.

Strychnine was given to 9 subjects in dosage of 3 mg. Only 2 showed an increase in after-contraction. Unlike caffeine, in every case where depression had been induced, strychnine failed to offset it.

In this report it is shown that of the three depressants studied, the bromide in all instances abolished or markedly reduced the after-contraction, whereas chloral and barbital, as a rule were ineffective. Of the 2 stimulants neither in the majority of instances produced any significant increase in the after-contraction. After it had been abolished by a depressant, caffeine brought the after-contraction back to its original state, strychnine was ineffective. These experiments throw additional light on the selective action of drugs on the central nervous system, and with others in progress should aid in the study of some of the specialized functions of the cerebrum.

8895 P

Effect of Liquid Air Temperature on Bacteria.

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1. Twenty-four-hour cultures of *B. typhi*, *B. coli*, *Staph. albus*, *B. subtilis* on agar slants and in beef tea were immersed in liquid air. In addition, strips of sterile filter paper that had been impregnated with the above cultures were also placed in liquid air. At the end of one week, the cultures and the filter paper were removed from the liquid air. They were then transferred to new media. After incubation all tubes showed growth.

2. A suspension of a 24-hour culture of *B. typhi* was made in physiologic saline solution and standardized to one million bacteria per cc. by cytometric count. Five cc. of the suspension were placed in sterile test tubes 150x13 mm. The tubes were sealed in a blast-lamp and then placed in liquid air. Daily, for a period of 10 days, a tube was removed and melted and the number of survivors de-

termined by the McCrady most-probable-number method, using 3 tubes of beef tea for each dilution. There was a drop from one million to 10,000 bacteria per cc. at the end of one day. The succeeding days gave the same count of approximately 10,000 per cc.

3. The technic was the same as in 2. At monthly intervals a tube was removed and the number of survivors was determined by the most-probable-number method, and by the plate-count. This was continued for a period of 13 months and further counts were made after 16 and 19 months.

The plate-counts and the most-probable-number sometimes agreed and at other times disagreed widely. There was shown no consistent decrease in bacterial numbers over the period of 19 months. The counts varied from 2.5 per cc. to 9500 per cc. over the period. The difficulty in getting consistent counts was apparently due to this: In the process of freezing, the liquid in the tubes freezes from the bottom up; as ice crystals form, the bacteria are pushed toward the surface and finally there is a layer of frozen bacteria at the surface. On standing this film becomes tough and a uniform suspension of bacteria is not obtained when the contents of the tube are melted and shaken.

Saline suspensions of bacteria that survive the mechanical effects of freezing are still viable after 19 months at about 83° absolute.

8896 P

A Study of Milk Coagulation as a Differential Feature of *Monilia albicans* and *Monilia candida*.

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Wisconsin.

Though *Monilia albicans* and *Monilia candida* have so far been shown to give the same serological reactions,¹ nevertheless in disagreement with Stone and Garrod's conclusion,² we consider^{3, 4, 5, 6}

* This work has been supported in part by a grant from the University Research Fund.

¹ Almon and Stovall, *J. Infect. Dis.*, 1934, **55**, 12.

² Stone and Garrod, *J. Path. and Bact.*, 1931, **34**, 429.

³ Stovall and Bubolz, *J. Infect. Dis.*, 1929, **45**, 463.

⁴ Stovall and Bubolz, *J. Infect. Dis.*, 1932, **50**, 73.

⁵ Stovall and Bubolz, *J. Lab. and Clin. Med.*, 1933, **18**, 890.

⁶ Stovall and Pessin, *J. Clin. Path.*, 1933, **3**, 347.

that there are sufficient cultural, biochemical, and pathogenic differences to justify placing the organisms in 2 different species. A prominent difference is the coagulation of milk by *M. albicans* in striking contrast to *M. candida*'s inability. The purpose of this study was to determine the conditions under which coagulation occurred and to see if in all cases a constant, definite difference in the species was observable. In addition, another objective was to demonstrate that the rennet-like coagulation of the *albicans* type is probably distinct from the adherent mat or mycelial pad formation of *M. candida* which produces a pseudo-coagulative effect.

In the work with coagulation the procedure for the preparation of the calcium-lactate milk medium is that formerly described.³

While it was found that coagulation could readily be accomplished through the use of any of the *M. albicans* cultures themselves yet the utilization of an extract from that species would permit a better comparison of clotting results with commercial rennin. Now in order to perform experiments with extracting agents it was necessary to obtain a heavy cell yield, and for this purpose large Blake bottles containing Trommer's malt-extract agar were used. After several extracting agents had been employed without marked success, sterile distilled water in 20 to 25 cc. portions per Blake bottle was used to wash down the growth. The suspension, kept at room temperature, was shaken at intervals and centrifuged at the end of a week. When 1 cc. of the clear supernatant was added to 10 cc. of the calcium-lactate milk, a coagulative effect was observed in less than 4 to 5 hours. The usual time heretofore was 2 to 3 days. Acid coagulation was ruled out, for electrometric pH determinations showed no change upon addition of the extract. Since the filtered *M. albicans* product induced clotting, the presence in solution of a coagulating agent free from cells seemed certain.

Other preparations were subsequently shown to contain the coagulating enzyme in even greater amount. Desiccated macerated cells yielded a potent preparation clotting of milk occurring within a few minutes after addition of the centrifuged supernatant. In addition the filtered liquid from a 4 weeks' growth in 75 cc. of malt-broth displayed very pronounced coagulative action in the *albicans* but not in the *candida* cultures.

Having obtained in solution an agent from *M. albicans* which clotted milk, we determined analogies which were establishable with a commercial rennet. The tests showed a striking relationship, both in manner and degree, of the factors affecting the extract and the commercial rennin; for example, calcium lactate added to sterilized milk rendered the medium readily coagulable by both products;

increasing the acidity of the milk shortened the coagulation time for both preparations, or rendering the milk alkaline prevented clotting, with the amount of extract and rennin regularly employed; adding ammonium oxalate, with resulting precipitation of the calcium together with a slight increase in pH, had an unfavorable effect for both products; and finally, a boiling temperature for 5 minutes rendered each entirely incapable of causing coagulation.

The effect of environmental conditions on the ability of growing organisms to produce clotting was studied. In each case in which an alteration in the milk was made, a decided effect was exerted on the *M. albicans'* ability or time to produce coagulation. Moreover, if the change was such that either rennin or the *Monilia* extract would be inhibited in its clotting action, then the live inoculum would likewise be affected. Similarly, changes more favorable for rennin action also rendered the milk more coagulable by growing organisms.

pH determinations, made at daily intervals, showed little change in reaction with the *M. albicans* cultures at the time of coagulation. However, at this time the *M. candida* displayed decided increase, a rise from pH 6.05 to 6.50 in 3.5 days, and a pH 7.4 in 8 days. The combination of reaction change together with *candida*'s marked deficiency of the coagulating enzyme is considered responsible for its failure to exhibit coagulation.* With the *M. albicans* a pH rise begins to occur after coagulation doubtless due to deaminization of protein material. However, even at the tenth day pronounced pH differences still obtain between the two species. That there is a definite biological difference between *Monilia albicans* and *Monilia candida* is shown on the basis of milk coagulation.

8897 P

Effect of 4-6 Dinitro-O-Cresol on Oxidation of d'- and l'-Arabinose by Previously Starved Yeast.*

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It has been shown in previous papers from this laboratory that the respiratory rate of yeast suspended in non-nutritive phosphate

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buffer solutions or in glucose phosphate can be markedly increased on treatment with proper concentrations of 2,4-dinitrophenol (DNP), 4,6-dinitro-o-cresol (DOC) and some related compounds.¹⁻⁵ We report herewith the results of experiments in which it is shown that the effects of such metabolic stimulants upon the respiration of yeast is profoundly affected by the type of "exogenous"⁶ carbohydrate fuel available.

A pure culture of *Saccharomyces cerevisiae* and pure culture methods were used throughout. The experimental procedures were the same as described previously^{2, 3} except that yeast was first grown 48 hours on Orla-Jensen agar medium, then taken up in 0.2M phosphate buffer, pH 6.8, and starved 24 hours under aerobic conditions in Fraser tubes⁷ at $25^{\circ} \pm 0.02^{\circ}\text{C}$. before the beginning of each ex-

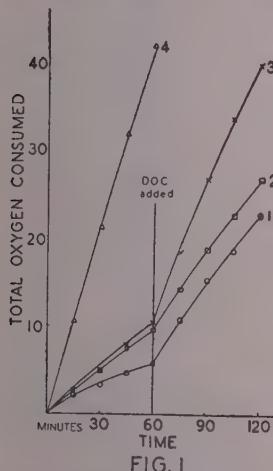


FIG. 1

Graph showing total amount of oxygen consumed in cmm. at N.P.T. per 10^8 cells as a function of time. Yeast suspended in 0.1M phosphate buffer, pH 6.8 (1), and in the same buffer with d'-arabinose (2), l'-arabinose (3) and glucose (4).

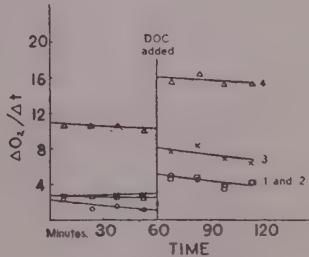


FIG. 2

Graph showing oxygen consumption in cmm. at N.P.T. per 10^8 cells as a function of time. Numbers of curves have same significance as in Fig. 1. Values of $\Delta O_2/\Delta t$ are for 15-minute intervals.

¹ Field, J., 2nd, Martin, A. W., and Field, S. M., PROC. SOC. EXP. BIOL. AND MED., 1933, **31**, 56.

² Field, J., 2nd, Martin, A. W., and Field, S. M., J. Cell. and Comp. Physiol., 1934, **4**, 405.

³ Field, J., 2nd, Martin, A. W., and Field, S. M., J. Pharm. and Exp. Therap., 1935, **53**, 314.

⁴ Field, J., 2nd, PROC. SOC. EXP. BIOL. AND MED., 1935, **32**, 1342.

⁵ Field, J., 2nd, and Martin, A. W., Compt. rend. Soc. de Biol., 1935, **119**, 458.

⁶ Stier, T. J. B., and Stannard, J. N., J. Gen. Physiol., 1936, **19**, 461.

⁷ Fraser, C. G., J. Physical Chem., 1921, **25**, 1.

periment. Respiration was measured in the conventional form of Warburg apparatus at $25^\circ \pm 0.02^\circ\text{C}$. Each set of quantitative comparisons was made on yeast from a single subculture. The observations reported represent typical findings in a series of 8 experiments involving 7 Warburg vessels each.

Fig. 1 illustrates integral curves showing the total amount of oxygen consumed in cu.m. at N.P.T. as a function of time when previously starved yeast is suspended in 0.1M phosphate buffer, pH 6.8, or in the same buffer containing 1% glucose (for comparison), 1% d'-arabinose (levo-rotatory) or 1% l'-arabinose (dextro-rotatory).

In this experiment DOC was added from the sidearms of the Warburg vessels after a 60-minute control period. The strength of the DOC was such as to furnish a concentration in the Warburg vessel of $5.05 \times 10^{-4}\text{M}$ (giving $7.96 \times 10^{-6}\text{M}$ free acid at pH 6.8), which was found optimal in a series of preliminary experiments. After addition of DOC, the slope of the integral curves in the presence of glucose (not shown in Fig. 1, but can be inferred from Fig. 2) and of l'-arabinose is greater, in the presence of d'-arabinose the same, as in the non-nutrient control. Since the integral curves for the two pentoses practically coincide before addition of DOC and draw apart afterward (Fig. 1), this finding constitutes an interesting case of stereoisomeric preference on the part of the yeast cell.

Fig. 2 illustrates differential curves showing oxygen consumption as a function of time in the same experiment. It is shown that the increase in $\Delta O_2 / \Delta t$ consequent upon addition of DOC in optimal concentration is definitely greater in glucose, slightly greater in l'-arabinose and definitely less in d'-arabinose than in the non-nutritive control. These results suggest that under the conditions of these experiments there is a respiratory "ceiling" for any given fuel. The action of metabolic stimulants, which increases $\Delta O_2 / \Delta t$ is limited by the position of this ceiling as well as by the properties of the stimulant.⁸ Further work on this point is in progress.

⁸ Field, J., 2nd, Martin, A. W., and Field, S. M., Proc. Soc. Expt. BIOL. AND MED., 1936, **34**, 388.

Effect of Vitamin C Administration on Vitamin C of Milk and Urine of Lactating Mothers.

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The relation of the vitamin C intake and the urinary excretion of the vitamin has been recently studied by a number of observers.¹⁻⁴ But little could be found in the literature regarding the effect of the diet on the vitamin C content of human milk. It is generally agreed that human milk is richer in the antiscorbutic vitamin than cow's milk and that the clinical manifestation of scurvy is very rare in breast-fed infants. In the case of cow's milk its vitamin C content is said to remain quite constant in different seasons despite the changes in the diet of the cow.¹ One may ask whether the human milk constantly provides an optimal amount of vitamin C regardless of the dietary supply of the same.

The present report is based upon the result of simultaneous determinations of vitamin C in the milk and urine of 2 lactating mothers for 26 and 27 consecutive days respectively. Their diet was adequate in the caloric intake but very low in vitamin C and consisted of food articles which were usually taken in the Chinese families of lower class. The diet was kept constant throughout except for a short period beginning on the fifth day of the study in which large doses of additional vitamin C substance were given. In case 1, the additional vitamin C consisted of 1000 cc. of canned orange juice ("Absopure") daily for 12 days; while in case 2, 600 mg. of ascorbic acid crystal (Schering-Kahlbaum) were given daily for 8 days. A total amount of 3500 cc. of fluids was allowed daily in each case. For the titration of vitamin C, the technique of Harris and Ray³ was followed closely. The output of vitamin C in 24-hour milk and in 24-hour urine was calculated and compared from day to day.

It was found that a sudden increase in the urinary excretion of vitamin C did not happen until the large dose of vitamin C substance had been given for 6 days in case 1 and for 3 days in case 2, showing that the storage of vitamin C in the tissue of these mothers

¹ Harris, L. J., Ray, S. N., and Ward, A., *Biochem. J.*, 1933, **27**, 2011.

² Johnson, S. W., and Zilva, S. S., *Biochem. J.*, 1934, **28**, 1393.

³ Harris, L. J., and Ray, S. N., *Lancet*, 1935, **1**, 71.

⁴ Youmans, J. B., Corlette, M. B., Akeroyd, J. H., and Frank, H., *Am. J. Med. Sci.*, 1936, **191**, 319.

was very low at the beginning of the experiment. Once the peak was reached the daily urinary output of the vitamin C tended to be fairly constant which amounted to about 60% of the intake. The excretion dropped abruptly with the discontinuance of the added vitamin in the diet.

Following the increase or decrease of vitamin C supply in the diet there was a corresponding fluctuation in the vitamin C content of milk, but the change was very slow and steady, being entirely different from the type of response in the urinary excretion. Milk, being a product of secretion rather than excretion, seemed to behave like the body tissue in this respect. After it had reached a "saturation" level which was around 0.08 mg. per cc. the concentration of vitamin C in the milk remained quite high for about 10 days even after the extra supply of the vitamin was discontinued.

8899 C

Effects of Acetyl Salicylic Acid on the General Condition and Blood Cells of Rats.

CHARLES J. ROBINSON, MILDRED ELLIS AND DOUGLAS WARNER.
(Introduced by C. H. Thienes.)

From the Department of Chemistry, Pomona College, and the Warner Laboratory, Claremont, California.

Published facts and opinions¹ regarding the low toxicity and safety of acetyl salicylic acid (aspirin) do not preclude the possibility that long continued daily use of this drug might be productive of bad results. This preliminary investigation, using white rats as subjects, was undertaken to test this possibility. The results were mainly negative.

Table I summarizes the results of administration of the drug to 33 young animals, 7 other animals constituting a control group. Weighed doses of acetyl salicylic acid were mixed with small portions of the standard mixed ration, and care was taken to assure that the entire dose was consumed each day. As compared with the control animals, the experimental animals were found to be normal in general physical condition, growth curves, appetite, activity, coat condition, and appearance of eyes, ears, tail and feces. Since doses of

¹ See esp. Editorial, *Lancet*, 1935, **220**, 619; Lowry, Otto, *Canadian Med. Assn. J.*, 1934, **31**, 638; Stoll, W. Fletcher, *Proc. London*, 1917, **99**, 293; Mueht, David L., *Med. Record*, 1918, **94**, 767.

20 to 50 gm. of acetyl salicylic acid are mentioned in the records of fatal poisoning of adult human beings, we assume that 20/70 or 0.28 gm. per kg. of body weight marks the beginning of the lethal range for human beings. It is noteworthy that the 4 animals in Group VI received daily doses in excess of this for 7 weeks without observed effects.

TABLE I.
Summary of Experiments with Rats Dosed with Acetylsalicylic Acid.

| Group | Period (beg. age, 5 wks.) | Daily dosage, gm./kg. | Human equivalent, 70 kg. body wt. | Results |
|-------|--|--------------------------|--------------------------------------|--|
| I. | weeks 25 | .022 | 1.54 23.8 | Normal |
| | 29 | .025 | 1.78 27.5 | " |
| II. | 15 | :022 incr. grad. to .11 | 7.72 119 | " |
| III. | 24 | .025 incr. grad. to .265 | 18.54 286 | " |
| IV. | 29 | .025 incr. grad. to .127 | 8.9 138 | " |
| V. | 25 | .084 incr. grad. to .408 | 28.5 440 | " |
| VI. | (At top level 1 week; in human lethal range 4 weeks) 9 (beg. age, 13 wks.) | .17 incr. grad. to .623 | 43.6 672 | " (In human lethal range for 7 weeks) |

Five animals unaccustomed to the drug were each given a single dose of acetyl salicylic acid equivalent to 39.6 gm. (611.4 grains) for 70 kg. body weight. They showed no toxic symptoms whatever. By mating rats which had been receiving heavy doses for 22 weeks perfectly normal litters were obtained. The treatment was continued throughout pregnancy.

Table II summarizes the results of blood cell counts of rats dosed for 14 weeks with .034 gm./kg. Since it has been shown by Warner² that the normal white rat shows a great deal of variation in white cell counts with variations in external temperature, light, food, water, sex activities and excitation by handling, and that a regular diurnal tide of considerable magnitude occurs, these conditions were regulated, and the diurnal tide was taken into account in interpreting the results. It is obvious that no substantial differences occur in the drugged animals as compared with the controls. Kerti³ found that in human beings, a decrease of about 10% occurred in the red cell count after 2-4 days of aspirin administration.

When the drug was withdrawn from the ration of rats which had become accustomed to it, no visible symptoms of disturbance occurred, but the blood cell picture was affected for several days.

In a group of 11 male rats receiving gradually increasing daily doses for 18 weeks, the average dose being 0.1 gm./kg., we

² Warner, Douglas, PROC. SOC. EXP. BIOL. AND MED., 1935, **33**, 230.

³ Kerti, Wiener klin. Wochenschr., 1929, **42**, 1630.

EFFECTS OF ASPIRIN ON RATS

TABLE II.

| Summary of Blood Cell Counts | Aver. of: | |
|------------------------------|---------------|------------|
| | 9 exper. rats | 8 controls |
| Total red count | 8,150,800 | 7,916,210 |
| Average deviation | 445,080 | 380,416 |
| Total white count | 14,551 | 14,493 |
| Average deviation | 3,495 | 3,275 |
| Distribution of white cells: | % | % |
| Lymphocytes | 66.5 | 72.7 |
| Monoocytes | 1.3 | 1.6 |
| Polynuclear neutrophiles | 24.7 | 20.9 |
| Metacytes | 3.8 | 3.2 |
| Staff cells | 0.9 | 0.7 |
| Polynuclear eosinophiles | 2.6 | 0.9 |
| Polynuclear basophiles | 0.4 | 0.2 |

find that the erythrocytes decrease somewhat on the second day after ceasing administration (perhaps due to blood dilution) return nearly to normal on the fifth day. The differential count shows increase of the polynuclear neutrophiles from a little over 20% to almost 40% on the second day, falling again gradually after 4 days; the metacytes and staff cells rise after 3 days, gradually fall on the fifth and sixth days; while the total leucocytes rise about 60% on the second day, return to normal about the sixth day.

Single doses failed to show substantial changes which could be attributed to the action of the drug. In such experimentation, it is necessary to establish the norm for each individual rat before giving the drug, and to anticipate the diurnal tide variations.

Summary. Daily dosage of white rats with acetylsalicylic acid for periods as long as 29 weeks, at high levels, produces no visible ill effects, and does not change the blood picture, except that a pronounced rise of white cell counts occurs on the second day after withdrawal of the drug from animals accustomed to it. It is obvious that the white rat is not suitable for testing the question of the possibility of ill effects in human beings from prolonged dosage with this drug.

8900 P

Simultaneous Excretion of Coproporphyrin I and III in a Case of Chronic Porphyria.

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In the urine and stool of normal individuals there is a small amount of coproporphyrin I present. In many pathological conditions, however, the amount excreted is considerably increased.^{1, 4} and in some conditions, notably lead poisoning, coproporphyrin III is excreted rather than the normal coproporphyrin I. Although it is probable that both types of porphyrins are produced in various diseases, the predominance of one type has made the isolation of both types difficult. In the case described below both coproporphyrin I and coproporphyrin III could be isolated from the feces of a woman suffering from chronic porphyria. Thus, the simultaneous formation of these 2 isomers is established.

The patient in whom this condition occurred was found in the psychiatric ward of the Strong Memorial Hospital. Since exogenous causes, such as lead and drugs, could be excluded, the condition is one of chronic porphyria. The patient was not anemic, did not suffer from abdominal colic and was not photosensitive; there was no evidence of abdominal bleeding. The patient was placed on a meat-free diet and the stool and urine collected. Because of the mental condition of the patient, collections were difficult to make.

The stool contained relatively large amounts of coproporphyrin, small amounts of a coproporphyrin ester and deuteroporphyrin and somewhat larger amounts of protoporphyrin. Only in the case of the coproporphyrin fraction was sufficient material present for identification. The porphyrin was methylated to the methyl ester and the product recrystallized from chloroform-methyl alcohol. After 3 recrystallizations crystals nearly uniform in appearance separated rapidly from the solvent. These melted indefinitely at 192°C. Further recrystallization raised the melting point to 194°C. and finally to 210-212°C. This behavior suggested a mixture of coproporphyrin I and III. The crystals were fractionated, therefore, by treating with cold methyl alcohol which dissolves coproporphyrin III methyl ester much more readily than it does coproporphyrin I

¹ Dobriner, K., *J. Biol. Chem.*, 1936, **113**, 1.

methyl ester. In this way 2 fractions, (A) methyl alcohol insoluble and (B) methyl alcohol soluble, were obtained.

A. *Methyl Alcohol Insoluble Fraction.* After 2 recrystallizations from chloroform-methyl alcohol crystals melting at 221-224°C. were obtained. Further recrystallizations, after each of which the crystals were washed with cold methyl alcohol, raised the melting point to 235-236°C. and finally to 238-239°C.

B. *Methyl Alcohol Soluble Fraction.* After 2 recrystallizations from chloroform-methyl alcohol the melting point was found to be 160-168°C. Two further recrystallizations in which crystallization took place with difficulty, as is characteristic of coproporphyrin III methyl ester, yielded a preparation with a double melting point, 135/170-175°.

The crystals of fraction A agree with the characteristics of coproporphyrin I methyl ester, m.p. 252°C., while fraction B is coproporphyrin III methyl ester, m.p. 142/172°C.

From the urine of this patient a very small amount of ether insoluble porphyrin (uroporphyrin) and a larger amount of ether soluble, chloroform insoluble porphyrin (coproporphyrin) could be isolated. The amounts present were too small for complete identification but the behavior of the ester of the coproporphyrin was suggestive of coproporphyrin III.

This appears to be the first instance in which simultaneous excretion of coproporphyrin I and III has been established. That both types of porphyrins may be excreted simultaneously has been shown by various investigators. Waldenström² isolated coproporphyrin I from the feces and uroporphyrin III from the urine of the same patient suffering from acute porphyria. In a case of lead poisoning, Watson³ has presented some evidence that coproporphyrin I is present in the stool while coproporphyrin III is excreted in the urine. Watson⁴ believes, also, that in normal urine there may be very small amounts of coproporphyrin III which have not been detected because of the extremely small amount of porphyrin, predominantly coproporphyrin I, which is present; his proof is admittedly incomplete. The uroporphyrin isolated from the famous case of Petry has recently been reexamined by Hans Fischer⁵ with the result that it could be separated into uroporphyrin I and III.

² Waldenström, J. Z. Physiol. Chem., 1936, **239**, III.

³ Watson, C. J., J. Clin. Inv., 1936, **15**, 456.

⁴ Watson, C. J., J. Clin. Inv., 1936, **15**, 327.

⁵ Fischer, H., and Libowitzky, H., Z. Physiol. Chem., 1936, **241**, 220.

8901 C

Ketosis Following Administration of Adrenal Cortex Extract.

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Following adrenalectomy the ketosis incident to the metabolic disturbances of fasting,¹ pancreatectomy,² phloridzin administration,³ pregnancy⁴ and administration of anterior pituitary extracts¹ does not occur. The mechanism by which the removal of the adrenals abolishes ketosis is not clear. If it is dependent upon a factor which operates through the adrenal cortex,⁵ and the production of ketosis by certain anterior pituitary extracts is obviously such a stimulus, either the presence of the adrenal cortex hormone in the organism is necessary or this tissue is stimulated to produce the ultimate ketosis factor. It seemed that the effect of adrenal cortex extract on ketosis might throw some light on this point, although not necessarily proving what mechanism is involved. Evans⁶ was unable to increase the urinary excretion of sugar and nitrogen in phloridzinized adrenalectomized animals by injection of a cortical adrenal extract. We have had similar results with one commercial extract ("Eschatin," Parke, Davis & Co.) which we used, but our dosage was high and some of the difficulties were definitely due to the phenol content (0.1%) of the preparation. With our own preparation we obtained indefinite results but another commercial product (Adrenal Cortex Extract, Wilson & Co.) which we have found more potent than either of these in maintaining the life of bilaterally adrenalectomized rats without salt therapy has influenced the ketosis of fasting rats quite definitely. The first adrenal cortex extract (Wilson) which we used contained no preservative. Later supplies contained 1:100,000 sodium ethyl mercurithiosalicylate (known under the trade name of "Merthiolate") but this preservative did not appear to have any effect on our results. Typical data are presented here.

The necessary information about each experiment is given in Table I. The rats were removed from the stock colony, where they

¹ MacKay, E. M., and Barnes, R. H., *Am. J. Physiol.* In press.

² Long, C. N. H., and Lukens, F. D. W., *Proc. Soc. Exp. BIOL. AND MED.*, 1935, **32**, 743.

³ Evans, G., *Am. J. Physiol.*, 1936, **114**, 297.

⁴ MacKay, E. M., and Barnes, R. H., *PROC. SOC. EXP. BIOL. AND MED.*, 1936, **34**, 682.

ADRENAL CORTEX EXTRACT KETOSIS

TABLE I.
Excretion of Ketone Bodies in mg. Per Rat Per Day.

| No. | Initial body wt., gm. | Day of Fasting | | | | |
|---|--------------------------|----------------|---------|---------|---------|---------|
| | | 1 | 2 | 3 | 4 | 5 |
| Experiment 1 (Adult female rats 500-600 days old) | | | | | | |
| Control Group | | | | | | |
| 1 | 241 | 16.8 | 17.3 | 14.9 | 1.4 | 2.5 |
| 2 | 227 | 2.5 | 8.1 | 38.6 | 11.4 | 2.4 |
| 3 | 216 | 4.6 | 15.3 | 34.9 | 13.8 | 4.9 |
| Av. | 228 | 8.0 | 13.6 | 29.0 | 8.9 | 3.3 |
| Adrenal Extract Group | | | (4 cc.) | (4 cc.) | (2 cc.) | |
| 4 | 235 | 2.8 | 75.4 | 154.0 | 121.0 | 140.0 |
| 5 | 234 | 2.2 | 16.2 | 136.0 | 63.0 | 6.9 |
| 6 | 225 | 3.1 | 61.2 | 150.0 | 94.6 | 113.0 |
| Av. | 231 | 2.7 | 50.9 | 146.6 | 92.9 | 83.3 |
| Experiment 2 (Adult male rats 240-300 days old) | | | | | | |
| Control Group | | | | | | |
| 1 | 250 | 1.6 | 0.7 | 1.2 | 0.7 | 6.6 |
| 2 | 206 | | 1.6 | 0.2 | 2.6 | 0.2 |
| 3 | 202 | 0.9 | 21.6 | 1.8 | 2.0 | 2.4 |
| Av. | 219 | 1.3 | 8.0 | 1.1 | 1.7 | 3.1 |
| Adrenal Extract Group | | | (3 cc.) | (5 cc.) | (5 cc.) | (5 cc.) |
| 4 | 230 | 2.3 | 23.0 | 11.5 | 4.0 | 2.2 |
| 5 | 220 | 1.1 | 4.6 | 11.0 | 11.7 | 28.9 |
| 6 | 208 | 2.5 | 19.5 | 15.1 | 9.7 | 1.7 |
| Av. | 219 | 1.9 | 15.7 | 12.5 | 8.5 | 10.9 |
| Adrenal Gland Emulsion | | | (4 cc.) | (4 cc.) | (4 cc.) | (4 cc.) |
| 7 | 223 | 2.5 | 13.2 | 1.0 | 1.1 | 1.5 |
| 8 | 230 | | 10.2 | 3.7 | 1.9 | 2.4 |
| 9 | 217 | 4.3 | 9.0 | 1.5 | 0.3 | 4.1 |
| Av. | 223 | 2.2 | 10.8 | 2.1 | 1.1 | 2.7 |
| Experiment 3 (Young adult female rats) | | | | | | |
| Control Group | | | | | | |
| 1 | 148 | | 1.5 | 2.2 | 2.6 | 4.2 |
| 2 | 168 | | 34.1 | 43.5 | 16.6 | 19.8 |
| Av. | 158 | | 17.8 | 22.8 | 9.6 | 12.0 |
| Adrenal Extract Group | | | (1 cc.) | (1 cc.) | (1 cc.) | (1 cc.) |
| 3 | 160 | | 24.0 | 19.1 | 21.0 | 12.3 |
| 4 | 163 | | 32.0 | 42.2 | 31.1 | 4.2 |
| Av. | 161 | | 28.0 | 30.6 | 26.1 | 8.2 |
| Experiment 4 (Young adult male rats) | | | | | | |
| Control Group | | | | | | |
| 1 | 200 | | 2.5 | 2.9 | 3.6 | 2.5 |
| 2 | 194 | | 4.3 | 2.4 | 1.6 | 1.5 |
| Av. | 197 | | 3.4 | 2.6 | 2.6 | 2.0 |
| Adrenal Extract Group | | | (1 cc.) | (1 cc.) | (1 cc.) | (1 cc.) |
| 3 | 219 | | 6.9 | 4.5 | 3.3 | 2.4 |
| 4 | 193 | | 3.1 | 4.9 | 4.5 | 1.8 |
| Av. | 206 | | 5.0 | 4.7 | 3.9 | 2.1 |

Experiment 5 (Young adult female rats, each given by stomach tube 200 mg. racemic sodium B-hydroxybutyrate per day)

| Control Group | | | 166.0 | 182.0 | 207.0 | 200.0 |
|---|-----|---------|---------|---------|---------|-------|
| 1 | 177 | | | | | |
| 2 | 161 | | 122.0 | 132.0 | 152.0 | 147.0 |
| Av. | 169 | | 141.0 | 157.0 | 179.5 | 173.5 |
| Adrenal Extract Group | | (1 cc.) | (1 cc.) | (1 cc.) | (1 cc.) | |
| 3 | 172 | | 127.0 | 220. | 239.0 | 209.0 |
| 4 | 161 | | 136.0 | 216.0 | 247.0 | 248.0 |
| Av. | 167 | | 131.5 | 218.0 | 243.0 | 228.5 |
| Control Group not fed ketone body (see control group of Exp. 3) | | | | | | |
| Av. | 158 | | 17.8 | 22.8 | 9.6 | 12.0 |

had been receiving our usual stock diet⁵ and fasting commenced. Water was allowed *ad lib.* Whenever adrenal cortex extract was given to one group the controls received an equal amount of physiological saline. All injections were made subcutaneously. Urine was collected under paraffine oil and the total ketone body content determined by Van Slyke's method.⁶

That the adrenal cortex extract used in these experiments increased the fasting ketosis is obvious from a perusal of Table I. Large doses had a much more definite influence (Exp. 1 and 2) than small doses (Exp. 3 and 4). The fasting ketosis of female rats (Control groups of Exp. 1 and 3) is greater than that of male rats (Control groups of Exp. 2 and 4). Adrenal cortex extract increases the ketosis of fasting female rats (Exp. 1 and to a less extent the lower dose Exp. 3) more than that of fasting male rats (Exp. 2 and to a less degree the low dose Exp. 4). The sodium salt of racemic B-hydroxy-butric acid is oxidized to some extent by fasting rats while the administration of adrenal cortex extract reduces this utilization so that it is negligible.

The ketogenic substance in the adrenal cortex extract may be the active hormone of this tissue or one of the group of miscellaneous substances from various sources which have the property of inducing or increasing a ketosis. We subscribe to the former view for a crude 25% emulsion of rabbit adrenals prepared by grinding them with a synthetic hydrated aluminum silicate powder ("Permutit"), which has the property of absorbing epinephrine, and injected at once, gave no increase in the degree of ketosis (Exp. 2). There is little reason to believe that this preparation contained an appreciable amount of cortical extract and in any case it did not increase the ketosis. Furthermore, some of the extract used in Experiment 1 stood in the laboratory for 6 months and then after refrigeration was overheated just before injection. It was then

⁵ MacKay, L. L., and MacKay, E. M., *Am. J. Physiol.*, 1927, **83**, 179.

⁶ Van Slyke, D. D., *J. Biol. Chem.*, 1917, **82**, 455.

inactive both in regard to its ketogenic activity and its ability to maintain the life of adrenalectomized rats.

Conclusions. An active adrenal cortex extract has been found to increase the ketosis of fasting female rats when administered in large doses. The ketosis of fasting male rats is only slightly increased. Adrenal cortex extract abolishes the partial oxidation of B-hydroxybutyric acid when fed to fasting rats.

8902 C

Effect of Allantoin Upon Fibroblasts from Cardiac Explants in Tissue Culture.

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It is difficult surgically and frequently impossible to eliminate all necrotic tissue and purulent materials from some deep wounds such as those of osteomyelitis.¹ To cope with this situation Baer² introduced the living maggot treatment which brought about early and complete healing of infections that had been resistant to other forms of accepted therapy. The beneficial results are possibly due to a combination of factors: (1) the maggots feeding upon and thus thoroughly removing all the diseased tissue; (2) the ingestion and physical removal of the microorganisms, with subsequent sterilization of the wound; (3) the proteolytic activity of the maggots' enzymes breaking down the discharge and slough of the wounds into their end products; (4) the maggots crawling about in the wound, causing sufficient irritation to stimulate rapid growth.³ In addition to these possibilities, it was believed that the larvae secreted some substance which stimulated directly the healing process.

Following investigations of the secretion of maggots, a substance was found which had the property of stimulating healing in infected wounds.⁴ It was identified as allantoin, the principal terminol product of purine metabolism in animals below man. It seemed evident that the secretion of this substance into the wounds contributed to the remarkable healing effects obtained by maggot therapy; how-

¹ Meyers, J., and Czaja, L. M., *Illinois Med. J.*, 1931, **60**, 124.

² Baer, William S., *J. Bone and Joint Surg.*, 1931, **13**, 438.

³ Buchman, Joseph, *Ann. Surg.*, 1934, **99**, 251.

⁴ Robinson, William, *J. Parasit.*, 1935, **21**, 354.

ever, allantoin, alone, could not be substituted for the living larvae. In its new rôle, as a stimulator of tissue growth where development is inactive, allantoin and some of its related substances appear to be more than waste products only. It was suggested that these substances might be used normally in the nuclear structure of the cell.⁵

The following experiment was devised and conducted to determine the effects of allantoin upon the growth and the rate of growth of cells in tissue culture.

All the cultures were made by the cover-slip-hanging-drop method with observance of aseptic precautions during all manipulations. All water used was triply distilled in a pyrex glass apparatus. The plasma was obtained by centrifuging blood drawn, without the use of anticoagulants, from the wing veins of young hens. The embryo juice (25%) for the control cultures was prepared by extracting 7- or 8-day chick embryos in Tyrode solution (pH 7.5 to 7.6) containing 0.25% dextrose. For the test series allantoin, in quantities sufficient for a final dilution of 0.5%, was added to an aliquot part of adjusted Tyrode solution before the extraction of the embryos. This concentration of allantoin was the same as that used in the clinical treatment of deep infected wounds, as reported by Robinson.* Heart tissue of 7- or 8-day chick embryos was planted in a mixture of equal parts of embryo juice, containing the allantoin, and plasma. An equal number of controls using the same plasma, stock embryo juice, and embryo heart, were planted at the same time with each series of allantoin cultures. All cultures were incubated at 37°C. and a daily record was kept of each as long as it remained alive. From these data on the control and allantoin cultures, average percentage death rate curves were constructed. With the aid of a delineascope and planimeter the area of the original explant and area of daily outgrowth of each culture were measured and the results statistically treated. (Table I.)

Microscopical study of each of the 560 cultures showed no marked difference in behavior or change in structure of the cells in either the control or experimental series. According to Graph 1, the death rate curves of both series of cultures followed practically the same course for the first 6 days, after which the curve of the allantoin series indicated a definitely greater death rate. This is in accord with the observation from Graph 2 that after the 5th day the growth of cultures in the allantoin medium was slightly increased and with increase in cell population nutrient materials were correspondingly diminished leading to an increased death rate.

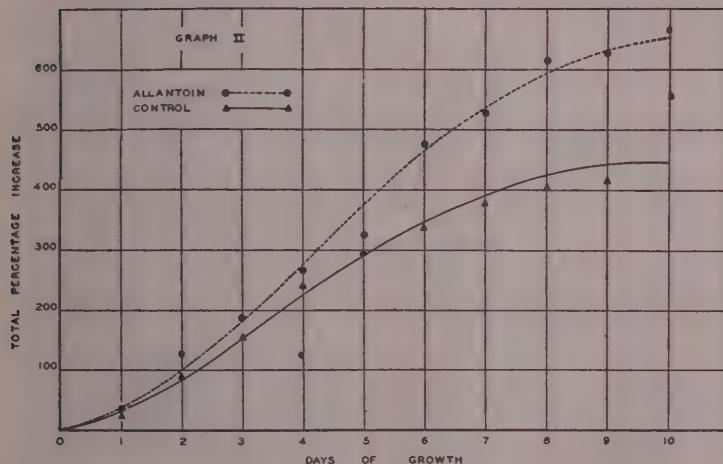
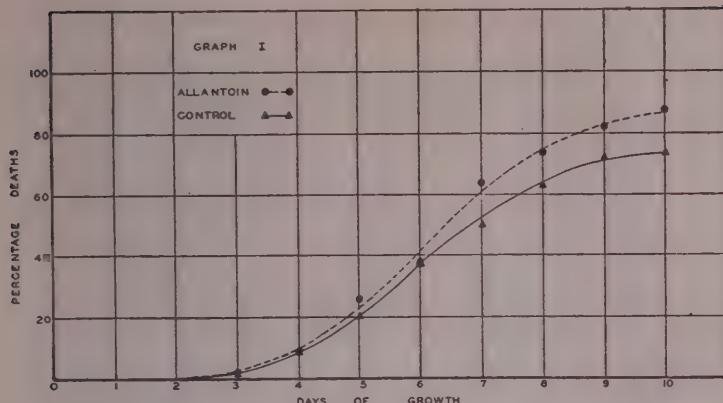
* Robinson, William, *J. Bone and Joint Surg.*, 1935, **17**, 267.

EFFECT OF ALLANTOIN UPON FIBROBLASTS

TABLE I.

| | Allantoin 0.5% Series | | | | | | Control Series | | | | | | |
|----|-----------------------|------------|------------------------|--------------------|---------------------------|----------------|----------------|------------------------|---------------------------|----------------|---------------------------------|-----------------------|--------------|
| | Mean Growth sq.mm. | Total Area | Probable Error of Mean | Standard Deviation | No. of Cultures in Exper. | Total Deaths % | Total Growth % | D—Difference of Means* | No. of Cultures in Exper. | Total Deaths % | Probable Growth Area in sq. mm. | Mean Deviation Exper. | |
| 0 | 4.21 | ±0.061 | ±1.46 | 270 | 0 | 1.00 | 0.17 | 1.00 > 0.51 | 5.21 | ±0.094 | ±2.45 | 280 0 | |
| 1 | 5.64 | ±0.088 | ±2.07 | 270 | 34 | 0.82 | 0.21 | 0.82 > 0.63 | 6.46 | ±0.108 | ±2.78 | 280 24 0 | |
| 2 | 9.67 | ±0.196 | ±4.48 | 270 | 129 | 0.23 | 0.38 | 0.23 < 1.14 | 9.90 | ±0.182 | ±4.50 | 280 90 0 | |
| 3 | 12.16 | ±0.274 | ±6.40 | 264 | 189 | 2.2 | 1.07 | 1.07 < 1.59 | 13.23 | ±0.256 | ±6.31 | 275 152 17 | |
| 4 | 15.33 | ±0.351 | ±8.03 | 247 | 264 | 8.5 | 2.39 | 0.71 | 2.39 > 2.13 | 17.72 | ±0.334 | ±7.99 | 255 240 8.9 |
| 5 | 17.86 | ±0.472 | ±9.50 | 199 | 324 | 25.9 | 2.79 | 1.01 | 2.79 < 3.03 | 20.65 | ±0.512 | ±11.31 | 223 296 20.3 |
| 6 | 24.37 | ±0.735 | ±14.11 | 167 | 478 | 31.8 | 1.73 | 1.48 | 1.73 < 4.44 | 22.64 | ±0.729 | ±13.27 | 175 334 37.5 |
| 7 | 26.45 | ±0.951 | ±13.93 | 97 | 538 | 64.0 | 1.62 | 1.84 | 1.62 < 5.52 | 24.83 | ±0.803 | ±13.96 | 139 376 50.3 |
| 8 | 30.02 | ±1.577 | ±17.52 | 65 | 613 | 75.9 | 3.86 | 2.68 | 3.86 < 8.04 | 26.16 | ±0.984 | ±14.92 | 105 402 62.5 |
| 9 | 30.50 | ±1.878 | ±16.42 | 48 | 624 | 82.2 | 3.79 | 3.18 | 3.79 < 9.54 | 26.71 | ±1.440 | ±18.69 | 78 412 72.1 |
| 10 | 32.19 | ±2.037 | ±20.92 | 35 | 644 | 87.0 | 2.02 | 4.08 | 2.02 < 12.24 | 34.21 | ±1.380 | ±17.67 | 74 556 73.5 |

*See foot-note 1.



In Graph 2 it is also apparent that the trend of the growth curve for the allantoin series is noticeably away from that of the controls yet analysis of the data* from which the curves are derived

* Significant difference of growth was determined for any given day from the following formulæ:

$$\sigma_D = \sqrt{\frac{\sigma_1^2}{N_1} + \frac{\sigma_2^2}{N_2}} \text{ and } D > 3\sigma_D$$

where for any one day σ_1 and σ_2 are the standard deviations of the control and allantoin series respectively and N_1 and N_2 , the number of cultures; "D" represents the difference of the means and σ_D is the standard error of the difference.⁶

6 Mills, *Statistical Methods*, H. Holt & Co., 1930, p. 558.

indicates that at no time is the difference in growth rate of the 2 series sufficiently great to have a real significance.

Conclusion. Allantoin stimulates slightly but not to a significant degree the growth of fibroblasts from cardiac explants in tissue culture.

8903 C

Intramuscular Injection of Ascorbic (Cevitamic) Acid and Excretion in the Sweat.

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The value of the oral and intravenous methods of administration of cevitamic acid has been well established in the treatment of vitamin C undernutrition.¹⁻⁹ In order to determine the efficacy of the intramuscular route, a group of patients was given the substance by this method, and the cevitamic acid values of the blood and urine were followed. The patients selected were of varying ages and degrees of vitamin C saturation and were all afebrile. The solution used was made up by mixing one mole of cevitamic acid with one mole of sodium hydroxide and contained 50 mg. of the vitamin per cc. The pH is about 6.3. It has remained stable during the 3 months of the duration of this experiment.* The use of sodium bicarbonate for this purpose, just preceding injection, was suggested by Fisher and Leake.¹⁰

The cevitamic acid content of the urine of the 2 preceding 24-hour

¹ Schultzer, P., *Lancet*, 589, Sept. 9, 1933.

² Schultzer, P., *Acta Med. Scand.*, 1934, **81**, 111.

³ Schultzer, P., *Acta Med. Scand.*, 1934, **83**, 544.

⁴ Schultzer, P., *Acta Med. Scand.*, 1934, **83**, 555.

⁵ Schultzer, P., *Acta Med. Scand.*, 1935, **85**, 563.

⁶ Wright, I. S., *PROC. SOC. EXP. BIOL. AND MED.*, 1934, **32**, 475.

⁷ Wright, I. S., and Lilienfeld, A., *Arch. Int. Med.*, 1936, **57**, 241.

⁸ Dalldorf, G., and Russell, H., *J. A. M. A.*, 1935, **104**, 1701.

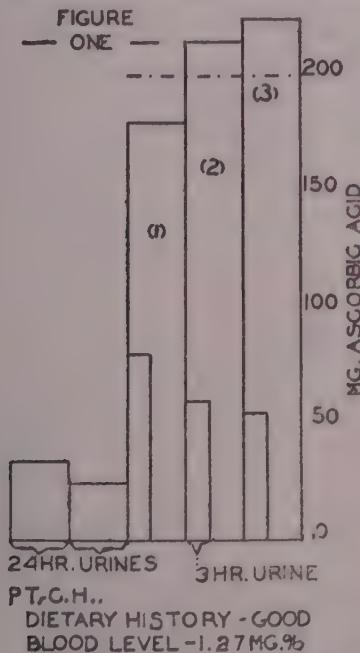
⁹ Van Eekelen, M., Over opname, Verbruik in Vitscheiding Van Vitamine C Door De Mens, Drukkerij Fa. Schotanus en Jens, Utrecht, 1936.

*Prepared and supplied through the courtesy of Merck and Co., Inc., Rahway, N. J.

¹⁰ Fisher, B. H., and Leake, C. D., *J. A. M. A.*, 1934, **103**, 1556.

periods¹¹ and the blood level of the vitamin^{12, 13} were determined as a control in each instance. Four cc. of the solution (200 mg.) was then injected into the gluteal region daily. Urine specimens were collected every hour for 3 hours and as passed for the remainder of the 24-hour period. This was repeated for 3 days, and, on the third day, 3 hourly blood specimens were taken after the injection, to determine the level of cevitamic acid in the blood plasma. It is important to note that the injection did not produce, on any occasion, evidence of local irritation or inflammation.

Effect on Urinary Excretion. Results. The results may be divided into 3 groups: 1. Cases in which the dietary history with respect to vitamin C was good and in which the initial values for 24-hour urinary excretion and blood level were high. Here, the first injection was followed by an immediate increase of cevitamic acid in the



Urinary excretion during control period (2 days) and following the injection of 200 mg. of ascorbic (cevitamic) acid intramuscularly daily for 3 days. Small columns represent urinary excretion during first 3 hours after injection. Typical curve following previous history of good vitamin C intake.

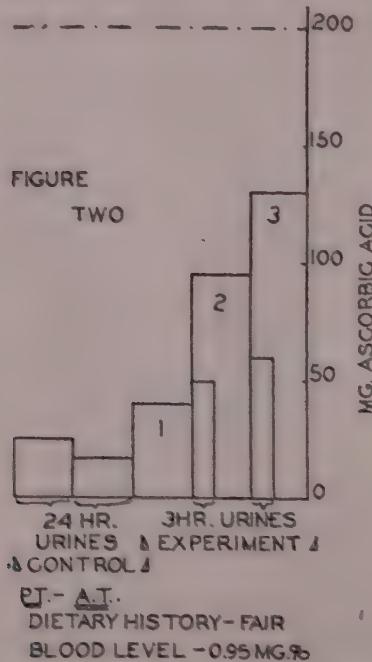
¹¹ Harris, L. J., and Ray, S. N., *Lancet*, **71**, Jan. 12, 1935.

¹² Abt, A. F., and Epstein, I. M., *J. A. M. A.*, 1935, **104**, 634.

¹³ Farmer, C. J., and Abt, A. F., *PROC. SOC. EXP. BIOL. AND MED.*, 1935, **32**, 1625.

urine. This increase was maintained as long as the injections were continued. A typical experiment with a patient (CH) in this group resulted in the following findings: original blood level, 1.27 mg. %; 24-hour urinary output of vitamin C: first control day, 38.6 mg.; second control day, 25.2 mg.; 24-hour urinary output after first dose of 200 mg. of Cevitamic Acid intramuscularly: 179.4 mg. (first 3 hours, 84.8 mg.); after second daily dose, 218.8 mg. (first 3 hours, 63.1 mg.); after third daily dose, 222.6 mg. (first 3 hours, 59.3 mg.).

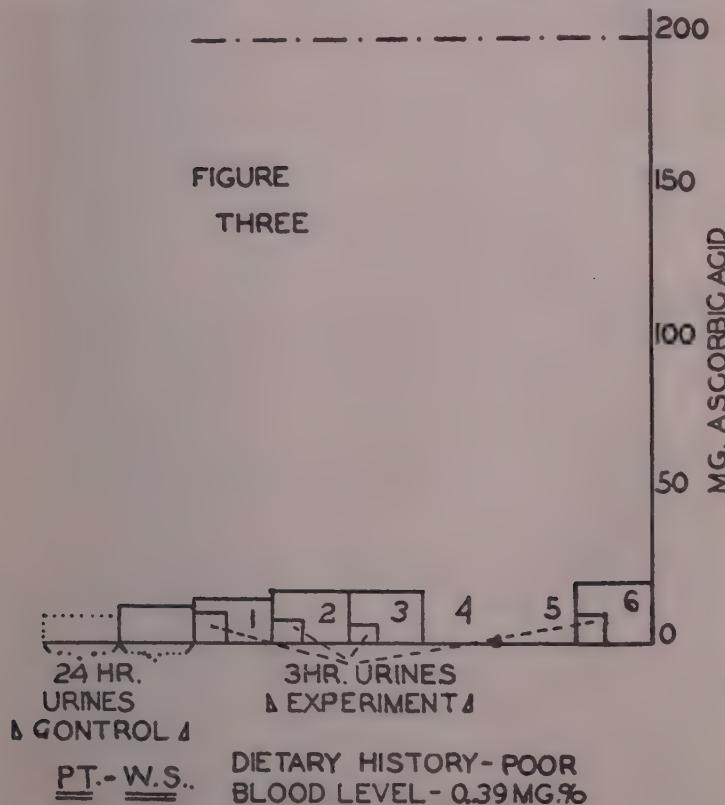
2. Cases in which the dietary intake of vitamin C had been only fair and in which the initial urinary and blood levels of cevitamic acid were correspondingly reduced. Here, an immediate increase in urinary excretion was still apparent, but it was not as sharp as, nor was it to the extent noted in group 1. (Fig. 2.) A typical experiment with a patient (AT) in this group resulted in the following findings: original blood level, .95 mg. %; 24-hour urinary output of vitamin C: first control day, 22.6 mg.; second control day, 16.1 mg.; 24-hour urinary output after first dose of 200 mg. of cevitamic acid intramuscularly, 39.2 mg. (3-hour studies not done); after second daily



Same of Fig. 1. Typical curve following previous history of fair vitamin C intake.

dose, 98.5 (first 3-hours, 42.56 mg.); after third daily dose, 135.2 mg. (first 3 hours, 38.4 mg.).

3. Cases in which the dietary history of vitamin C was frankly inadequate. The 24-hour urinary levels and blood plasma content of cevitamic acid were definitely low, and 3 days of the intramuscular injections did not suffice to raise the urinary excretion above the original values. In one patient who was followed for an additional 3 days, a slight increase was noted in the 24-hour urinary excretion of the latter period. A typical experiment with a patient (WS) in this group resulted in the following findings: original blood level, .39 mg. %; 24-hour urinary output of vitamin C: control day, 6.7 mg.; 24-hour urinary output after first dose of 200 mg. of cevitamic acid intramuscularly, 13.39 mg. (first 3 hours, 5.28 mg.); after second daily dose, 13.94 mg. (first 3 hours, 3.45 mg.); after third

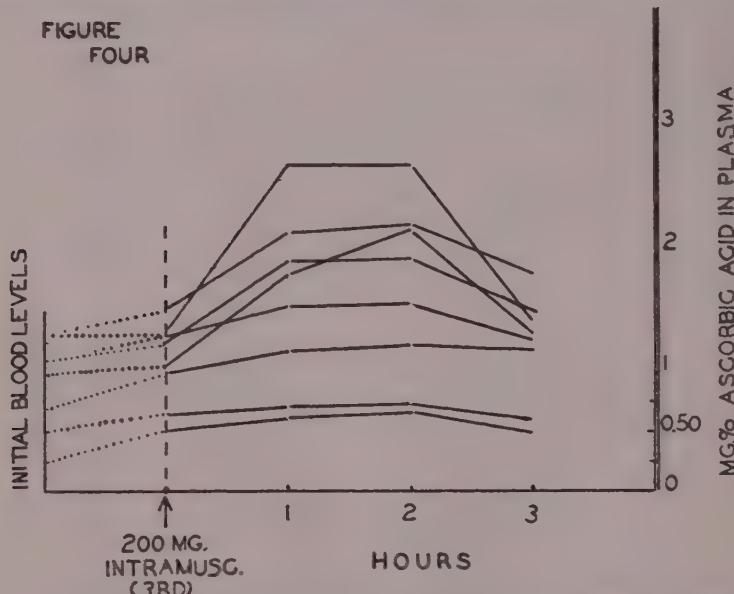


Same as Fig. 1 except that urinary excretion was checked on 6th day. Typical curve following previous history of poor vitamin C intake.

daily dose, 14.36 mg. (first 3 hours, 3.11 mg.); after sixth daily dose, 18.18 mg. (first 3 hours, 6.35 mg.).

Effect on Cevitamic Acid Level of Blood Plasma. In all 3 groups of vitamin C saturation, the blood level of cevitamic acid was increased, the height of the concentration being reached and maintained in the first and second hours and dropping rapidly in the third hour towards its original level. In 24 hours, the original level or a slightly higher one was reached. The rise in the first 2 hours was most abrupt in cases where the original nutritional state with regard to vitamin C was good. (Fig. 4.) The blood level was, however, influenced even in those cases of poor dietary history in which the 24-hour urinary excretion was not increased after 3 days of intramuscular injection of cevitamic acid.

FIGURE
FOUR



Curves showing the effect of the injection of 200 mg. of ascorbic (cevitamic) acid intramuscularly on its level in the blood plasma.

The height of rise of the blood level following the intramuscular injection of cevitamic acid was reached more slowly and was maintained for a longer period of time than after intravenous injection.

In one case, belonging in the group of fair vitamin C nutrition, there was an unexpected decrease in the 24-hour urinary excretion of vitamin C following the second injection (115 mg. after the first, 93 mg. after the second, and 165 mg. after the third). No portion

of the urine had been lost, as far as could be ascertained, and there was no increase in body temperature. During the day, however, the outside temperature had risen to 102°F., and the patient perspired profusely. The possibility was, therefore, suggested that significant amounts of cevitamic acid may have been lost through the skin in the sweat. We have since found that the sweat may contain appreciable amounts of vitamin C. This problem is being investigated at the present and will be reported upon shortly.

Conclusions. 1. Cevitamic acid, properly buffered, may be administered intramuscularly without discomfort or damage to tissue. 2. Studies of the urinary excretion and the blood content of vitamin C following the administration of cevitamic acid intramuscularly demonstrated that it is used by the body when administered by this route. 3. The height of increase in the blood level following the intramuscular injection of cevitamic acid is reached more slowly and is maintained for a longer time than after intravenous injections. 4. In cases of vitamin C deficiency, where cevitamic acid is improperly absorbed through the gastro-intestinal tract, or where the intravenous mode of administration is not feasible, the intramuscular route may be used.

8904 C

Variability of Metabolic Response of Different Children to a Given Intake of Calcium.

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As the metabolic balance studies on the "so-called normal" adults^{1, 2} and 22 children^{3, 4} as well as the growth observations on 530 infants⁵ have progressed in this laboratory over several years

¹ Macy, I. G., Hunscher, H. A., Nims, B., and McCosh, S. S., *J. Biol. Chem.*, 1930, **86**, 17.

² Hunscher, H. A., Donelson, E., Erickson, B. N., and Macy, I. G., *J. Nutrition*, 1934, **8**, 341.

³ Hunscher, H. A., Cope, F., Noll, A., Macy, I. G., Cooley, T. B., Penberthy, G. C., and Armstrong, L., *J. Biol. Chem.*, 1932, **97**, LXIV.

⁴ Hunscher, H. A., Cope, F., Noll, A., and Macy, I. G., *J. Biol. Chem.*, 1933, **100**, LV.

⁵ Unpublished data from this laboratory.

we have grown more and more impressed with the wide physiological variations found. These fluctuations are evident not only among individuals of the same age, body size and living under the same environmental conditions but of the same individual when kept under constant conditions and observed continuously over, not days, but weeks. Obviously, it becomes necessary to build up some knowledge as to how much variation one may expect to find in a healthy individual living under highly standardized conditions before it is possible to interpret the degree of differences of results found under specific experimental conditions or in disease. Moreover, in the case of children, it is exceedingly important to build up some concept of the changes that occur incident to growth and development alone, before one is able to interpret satisfactorily the significant effect of specific food or other amenable factors on the metabolism of the child.

To illustrate not only the degree of variability that may occur among healthy individuals of like age and body size but also the increments and decrements that occur in metabolism from time to time as the observations progress the present report records the calcium balances for only 25 to 65 consecutive days of 6 of the 22* typically healthy children who were maintained on simple diets of 70 to 100 calories per kilo of body weight per day appropriate for the age and size of the child and approximately uniform in mineral and nitrogen content. The dietary furnished one gram of calcium per day for each child.

The growing children under observation had excellent health records and were good eaters with no idiosyncracies. They had become accustomed to the routine management through a dietary control period preceding the study and were happy and interested in their wholesome regularity of personal habits, sleep, work, play in the fresh air and sunshine of the open country and in their healthful living in a home environment where love, security, and serenity abounded. These children were experts in *eating all the food* given to them, in the accurate collecting of urine and separating it from the feces. The study carried no strain for them but on the contrary gave them an opportunity to excel in a fete that gave them social prestige and admiration among their peers and brought praise from the adults in attendance. With as highly standardized a group of children as is humanly possible to secure where heredity and other physiological factors are uncontrollable, with living environ-

* These data are representative of those found in 540 five-day balances for acid-base minerals and nitrogen on 22 children, 10 of whom were observed for 225 continuous days. The complete study will be reported in detail later.

TABLE I.
Metabolic Response of 6 Children to the Same Calcium Intake.
(1 gm. per day).

| Sub- ject | Sex | Beginning Age | Height cm. | Weight kg. | Days Studied | Average Daily Retention | | | % Re- tained |
|--------------|-----|------------------|---------------|---------------|-----------------|-------------------------|----------------|----------------|-----------------|
| | | | | | | Total gm. | per kg. gm. | per cm. mg. | |
| 5-Year. | | | | | | | | | |
| D.P. | M | 4-9-3 | 107.5 | 19.2 | 55 | 0.48 | 0.024 | 4.4 | 48.5 |
| C.L. | F | 5-4-18 | 106.7 | 17.8 | 65 | 0.27 | 0.015 | 2.5 | 27.4 |
| P.S. | M | 5-5-23 | 114.8 | 22.3 | 30 | 0.31 | 0.014 | 2.7 | 31.2 |
| B.C. | M | 5-8-15 | 111.8 | 19.6 | 30 | 0.32 | 0.016 | 2.8 | 31.5 |
| 8-Year. | | | | | | | | | |
| S.C. | F | 8-2-3 | 125.1 | 24.2 | 25 | 0.48 | 0.020 | 3.8 | 47.8 |
| J.L. | F | 8-10-4 | 128.3 | 25.3 | 30 | 0.29 | 0.011 | 2.2 | 29.0 |

ment satisfactory from the scientific point of view, and stimulating happiness as well as serenity for all subjects alike, the results that follow should be of significance in the use of the balance method for determining dietary requirements.

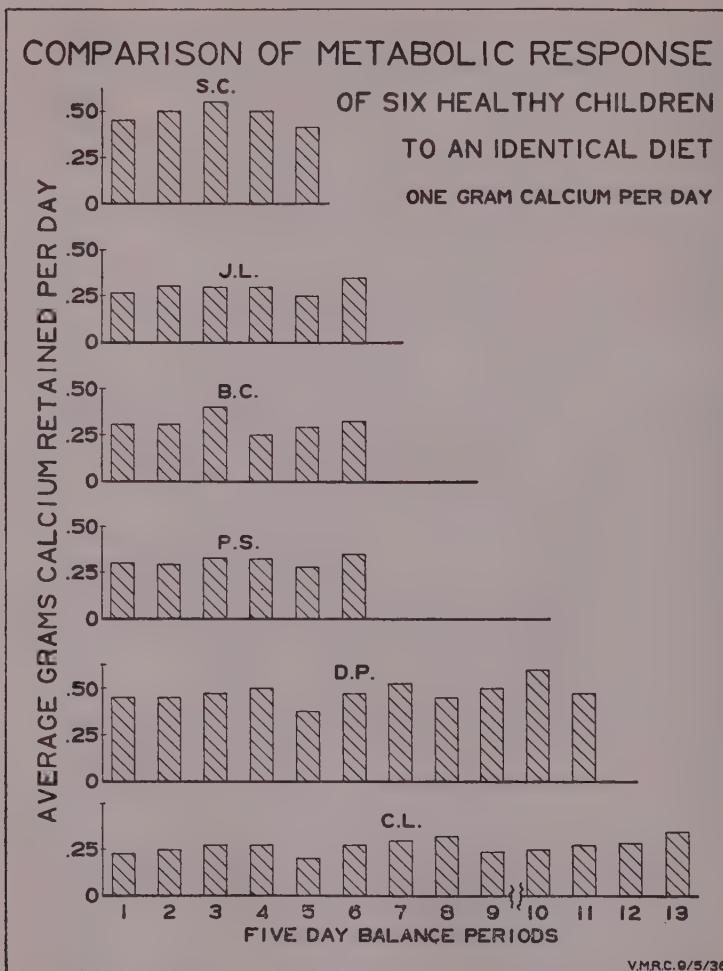
Table I shows the individual metabolic response of 6 children to a dietary intake of one gram of calcium per day. Over an observation period of 30 to 65 consecutive days 4 five-year-olds showed an average total daily retention of calcium ranging from 0.27 to 0.48 gm. and varying from 27.4 to 48.5%. When the average daily storage of calcium is interpreted in terms of body size the individual differences are just as great, varying from 0.014 to 0.024 gm. per kilo body weight and 2.5 to 4.4 mg. per cm. body stature.

Age does not explain these typically wide individual differences in response of children as illustrated by the inclusion and comparison of the metabolic records of 2 eight-year-olds with the 4 five-year-olds in Table I. They, too, showed similar levels of daily retentions of calcium on the same dietary intake but the range of difference is just as great, namely 0.29 to 0.48 gm. per day. Likewise, when body size is considered the same individual trend is evident in all 6 children.

Chart 1 is illustrative of the increments and decrements of storage of calcium that may be observed in many healthy individuals¹⁻⁷ maintained under strict metabolic conditions, provided they are followed continuously over a considerable period of time. It has been previously pointed out that the study of consecutive balance periods not only reveals individualities in the response for acid-base mineral elements and nitrogen of different subjects to identical conditions but variations from period to period.

⁶ Clark, G. W., *Univ. Calif. Pub. in Physiol.*, 1926, 5, 195.

⁷ Porter, Levin T., *J. Am. Diet. Assn.*, 1933, 9, 22.



The physiological inconstancy of childhood is distinctively illustrated in Chart 1. Obviously it is necessary to learn throughout a pre-experimental period of several weeks how wide these customary physiological fluctuations may be for a specific child as well as the individual rates of storage under highly standardized and desirable conditions before the effect of any regimen upon the metabolic balance can be satisfactorily understood. Without the inclusion of such controlled data one may be easily misled into false fields of interpretation by the use of this method for determining the subsequent effect of certain foods or other factors on metabolism.

8905 C

Studies on Gonadotropic Antihormones.

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This work was originally begun with the idea of determining, if possible, the nature of the "inhibiting substance" which Collip *et al.*,¹⁻⁵ have described and designated as "antihormones." The controversy which has arisen involves the question as to whether the action of the "antihormone" may not in reality be due to an antibody developed against the protein, which is always found in hormone preparations derived from human or animal source. It is our belief that the gonadotropic inhibiting substance is separate and distinct from the antiprotein and both are present in the antiseraums obtained from rabbits which have been persistently injected with large doses of pituitary extract or P.U.* hormone.

One male and 3 female adult rabbits were injected over a period of 3 weeks, receiving 5 cc. of pituitary extract (E. R. Squibb and Sons) daily, totaling 900 growth units each. The antiserums, inactivated at 56° for ½ hour, gave a precipitin reaction with pituitary extract. On testing the pituitary extract for gonadotropic hormone, we found that 1/6 cc. injected intravenously into a female† rabbit gave corpora lutea. With the addition of 1 cc. of the antiserum to 1/3 cc. of pituitary extract, a heavy precipitate formed. The supernatant along with the precipitate was injected intravenously into an immature female rabbit. Examination of the ovaries 48 hours later showed no stimulation. Similar results were obtained with the other 3 antiserums.

We attempted to remove the anti-bovine protein present in our pituitary antiserums by treatment with normal bovine serum. Two cc. of bovine serum (1-100) incubated 2 hours with 2 cc. of pituitary antiserum gave a heavy precipitate which was centrifuged out.

¹ Collip, J. B., *J. Mount Sinai Hosp.*, 1934, **1**, 28.

² Collip, E. M., and Collip, J. B., *Lancet*, 1934, **1**, 784.

³ Selye, H., Bachman, C., Thompson, D. L., and Collip, J. B., *PROC. SOC. EXP. BIOL. AND MED.*, 1934, **31**, 1113.

⁴ Bachman, C., and Collip, J. B., *PROC. SOC. EXP. BIOL. AND MED.*, 1935, **32**, 554.

⁵ Bachman, C., *PROC. SOC. EXP. BIOL. AND MED.*, 1934, **32**, 851.

* P.U. = Pregnancy urine—gonadotropic fraction.

† Female rabbits 10 to 12 weeks of age and from a single source were used for testing purposes.

The supernatant when tested with bovine serum in dilutions up to 1-10,000 gave no precipitin reaction, indicating that the anti-bovine protein had been eliminated. Two cc. of antiserum so treated was incubated for 1 hour with 1/3 cc. of pituitary extract. The mixture was injected intravenously into a female rabbit and no ovarian stimulation was noted 48 hours later. This same result was obtained with each of the 3 antiserums so treated. Precipitation of anti-bovine protein thus does not remove the inhibitory factor (antihormone) from solution.

A mixture containing 1 cc. of pituitary antiserum and 15 rat units of antophysin[‡] (Winthrop Chemical Co.) was incubated 1 hour and then injected intravenously into a female rabbit. Forty-eight hours later, autopsy showed numerous corpora hemorrhagica in the ovaries, indicating that pituitary antiserum did not inhibit the action of P.U. under the conditions of the experiment.

In testing both antophysin and follutein[§] (E. R. Squibb and Sons) we found that 15 R.U. injected intravenously into immature rabbits gave a good Friedman test 48 hours later. Two female rabbits were each injected with 13,000 R.U. of antophysin and 2 other female rabbits were injected with 12,000 R.U. of follutein over a period of 3 weeks. Each antiserum gave a precipitin reaction with normal male human serum and with its own antigen. One cc. of antophysin antiserum was incubated one hour with 25 R.U. of antophysin and injected into an immature female rabbit. Autopsy 48 hours later revealed normal unstimulated ovaries. A similar result was obtained when follutein antiserum plus 25 R.U. of follutein was tested.

Two cc. of antophysin antiserum was incubated 2 hours with 2 cc. of normal male human serum (1-100). A heavy precipitate formed which was centrifuged out. Subsequent precipitin reactions between the supernatant and normal human serum in dilutions up to 1-10,000 were negative, indicating that the anti-human protein factor had been removed. Two cc. of this treated antiserum was incubated 1 hour with 25 R.U. of antophysin and injected intravenously into a female rabbit. No ovarian stimulation was noted 48 hours later.

The above experiment indicates that after absorption of the antiserum with human serum protein, a factor remained in the supernatant fluid which was still capable of inhibiting the action of the hormone used to produce this antiserum. This was not due to the

[‡] Antophysin = P.U. extract.

[§] Follutein = P.U. extract.

antiprotein factors since, as far as we could determine these had been removed by absorption.

In testing the action of antophysin antiserum on pituitary extract, 1 cc. of the antiserum was incubated 1 hour with 1/3 cc. of the extract and the mixture injected into a female rabbit. There was no ovarian stimulation 48 hours later. A similar result was obtained when follutein antiserum was tested against pituitary extract. The pregnancy urine *antiserum* inhibited the gonadotropic action of pituitary extract as well as P.U.; whereas pituitary antiserum (as shown above) inhibited the gonadotropic action of only the pituitary extract. We feel that there was not sufficient gonadotropic "antihormone" developed to inhibit the hormone action of P.U.|| Our assay of pituitary extract for gonadotropic hormone revealed 20 R.U./cc.; thus the rabbits injected with pituitary extract received only a total of approximately 1750 R.U. of gonadotropic hormone.

The fact that our P.U. antiserums, after treatment with human serum proteins, will still inhibit the hormone action of P.U. is a significant finding; and since these antiserums will also inhibit the hormone action of pituitary extract derived from bovine source, it thus appears that the antihormone (inhibitory action) is not due to an anti-human protein phenomenon *per se*, but to the presence of another antagonistic but specific substance.

8906 P

Cataleptic Symptoms Following Bilateral One-Stage Removal of Frontal Cortex in Cats.

RALPH W. BARRIS. (Introduced by E. B. McKinley.)

From the Department of Anatomy, George Washington University Medical School, Washington, D. C.

The author wishes to present a brief report concerning a peculiar group of symptoms, in many ways suggestive of catalepsy, which has been observed in 8 cats following a one-stage operation in which both frontal lobes of the cerebral cortex were removed. These operations were done with every precaution to secure good hemostasis and prevent hemorrhage from complicating the interpretation of the subsequent observations. Careful autopsies revealed that in no instance was there any sign of hemorrhage at the base of the brain

|| Recent unpublished experiments show that the pituitary antiserum as well as P.U. antiserums will inhibit the gonadotropic action of pregnant mare serum.

or of blood in the ventricles. The observations on animals (cats) were made on relatively acute preparations. Cats 5, 6, 7, 8, 9, 10, 11, and 12 survived for 28, 3, 19, 2, 8, 13, 6 and 15 days, respectively. Animal 9 died after a laboratory accident; animal 6 of pneumonia, and animal 8 of an infection contracted post-operatively. All others were sacrificed.

These animals all showed a remarkable plasticity of the muscles of the limbs and back. Such plastic tonus was especially well observed if the animals were placed in a supine position in a wooden trough-like structure. The limbs of these animals were usually rigidly extended when in this position. If they were passively flexed by the observer, there was considerable resistance, and the limbs remained flexed for exceptionally long periods of time, hours in many instances. When passively extended, there was likewise considerable resistance, and the limbs remained extended for very long periods. Furthermore, these animals made little or no effort to escape when in the supine position, but would lie quietly, breathing regularly. They were neither moribund or comatose. Moving objects placed before the eyes elicited a following movement of the eyes. Sounds caused a twitching of the ears.

These animals could walk within a day or two after the operation. All could stand 24 to 36 hours after operation. The general behavior of these animals was distinctly abnormal. All required feeding by tube in the early survival period, and in one instance (cat 12) tube-feeding was always necessary. These animals were apparently oblivious to such painful stimuli as severe pinching of the tail during the early survival periods. Such "reflex" responses, as hair erection or alteration of the respiratory rate were never observed in any of the animals no matter how severe the pinching of the tail might be. Pupillary dilatation of a moderate degree could be produced only in the later survival periods and at this stage only very weak movements of a defensive character could be produced by severe pinching of the tail.

A rather surprising characteristic of these animals was their great deficiency in the ability to right themselves when dropped from either side or back into an outstretched hammock. Such a reaction when present was never normal, but always delayed and incomplete. This is indeed peculiar in view of the remoteness of the labyrinthes and their sub-cortical neural pathways from the area of cortical injury.

One might suspect that many of the above symptoms as briefly mentioned here were due to an involvement of brain-stem structures; but although such an opinion is plausible, it is not likely that

this explanation is a valid one in view of the absence of any observable hemorrhage in this region or within the ventricles. Only 8 animals were operated; all 8 showed the same qualitative symptoms, there being only a slight quantitative difference. If such symptoms were due to hemorrhagic involvement of the brain-stem, it would seem remarkable that such a similar group of symptoms could be produced in 8 consecutive animals, without any evidence whatever of other symptoms such as rigidity of a decerebrate type or impairment of the respiratory mechanism. Furthermore, the nature of the symptoms was a constant finding in all animals regardless of their period of survival, although quantitatively there was some diminution in later stages.

8907 C

Stimulation of Birth-mechanism in Experimentally Monotocous and Oophorectomized Rats.

H. O. HATERIUS.

From the Physiological Laboratory, The Ohio State University.

It was demonstrated recently¹ that ovarian ablation in the rat during the last third of pregnancy need not interrupt this condition, providing the litter being carried has been surgically reduced to one fetus and that the placentae of the fetuses removed have been allowed to remain intact. Under these circumstances, however, the animals fail to deliver and the young, to be obtained alive, must be recovered by operative procedure; retention *in utero* much beyond the normal time of parturition serves progressively to compromise their viability. It seems improbable that failure of birth is due to the manipulations involved since control animals, receiving similar treatment save for retention of one ovary, will litter at the usual time. In failure of the birth mechanism the rat appears to differ from other species reported to carry to term following upon oophorectomy; the guinea pig, for example, spayed during the latter half of pregnancy, frequently continues to term and to normal delivery;² substantially the same situation prevails for the mare³ and, as is well known, for the human being.⁴

¹ Haterius, H. O., *Am. J. Physiol.*, 1936, **114**, 399.

² Nelson, W. O., *Endocrinol.*, 1934, **18**, 33.

³ Hart, G. H., and Cole, H. H., *Am. J. Physiol.*, 1934, **109**, 320.

⁴ Ask-Upmark, M. E., *Acta obst. et gynec. Scand.*, 1926, **5**, 211.

The marked rigidity and contraction of the uterus in cases of uninterrupted pregnancy suggest either that it has become insensitive to, or that it lacks, the stimuli involved in the processes of labor. Since oestrin serves as a powerful, and in certain respects a specific, sensitizer of uterine tissue⁵ it seemed probable, in the absence of ovaries, that deficiency in this principle might be an underlying factor in failure of birth. The present communication is concerned with the results of attempts at testing this possibility.

Female rats, prepared by unilateral tubal resection, were mated; resulting pregnancies were carefully timed and at laparotomy upon the 12th-13th day all fetuses in the fertile horn in excess of one were removed, the placentae being left *in situ*; in addition one ovary was extirpated. Uniformly upon the 15th day the remaining ovary was ablated. As reported elsewhere rats so prepared will usually carry to term but will fail in delivery, although the young may be recovered through timely surgical intervention.

In preliminary work, following upon complete oophorectomy, single, rather large doses of oestrin (Amniotin*) were administered as the animals approached term. In 7 cases dosages of 500 R.U. on day 20 or 21 of gestation failed to evoke a response. In 5 instances 2 daily doses of 500 R.U. produced no effect, beyond the appearance of cornified smears, and dead, somewhat macerated fetuses were recovered surgically upon the 24th day. It appeared that the normal course of events might more readily be simulated by daily administration of smaller quantities (25 and 50 R.U.) beginning soon after oophorectomy. Results were negative, however, in 12 animals employed. It was only when the daily dosage, although comparatively small, was markedly increased from day to day that a response at all resembling parturition was elicited, and the present report deals with results obtained in 5 series treated in this manner.

Series A. Commencing upon the day following complete oophorectomy, oestrin was administered in 3 daily injections in an effort to replace so far as possible the presumptive oestrin loss occasioned by ovarian removal. The dosage was arbitrary, for lack of accurate data, in this series being progressively increased, *i. e.*, 16 R.U. on the 16th day, 24 on the 17th, 32 on the 18th, 40 on the 19th, 48 on the 20th—a total of 160 R.U. over a 5-day period. Treatment was suspended after the 20th day. Considerable variation in response was encountered, 4 animals aborting upon the 19th day, 3 upon the 20th, and 3 carrying to term but failing in delivery; one

⁵ Reynolds, S. R. M., *Am. J. Obst. and Gynec.*, 1935, **29**, 630.

* The writer is indebted to Dr. J. A. Morrell, of E. R. Squibb and Sons, for generous supplies of Amniotin.

fetus was recovered alive, but deformed and not viable, on the 24th day. The fetuses aborted either were eaten or were recovered dead; one aborted on the 20th day was alive but died shortly after delivery.

Series B. In the second group the original dosage was doubled (32, 48, 60, 80, 96 R.U. upon successive days of the 5-day period). These all aborted on the 19th and 20th days, the vaginal smears displaying cornified cells mixed with leucocytes and blood from the third day onward. Two dead fetuses were recovered, the remainder apparently having been eaten.

Series C. Attempts were made to give a dosage such that 50% of the animals would abort, allowing the remainder to carry to the 21st day. Fifteen animals in this group were given 24, 36, 48, 60, 72 R.U. on successive days, treatment being suspended after the 20th day. Five animals aborted on the 19th and 20th days, displaying cornified and bloody smears. Ten animals carried through and were sacrificed on the 23rd day. From these 4 viable young were recovered, 3 living but not viable, 2 dead; the uterus of the remaining animal had been recently emptied.

Series D. (Table I.) The same dosage was employed in this series with the exception, however, that treatment was continued beyond the 20th day, the non-responsive animals receiving 96 R.U. on the 21st day and 120 P.U. on the 22nd. Of 12 animals used, 4 aborted on days 19 and 20, 3 on the 21st, and 3 on the 22nd day. Two of the young recovered on the 22nd day were viable and were reared by foster mothers. The remaining two of this series failed

TABLE I.
Influence of Oestrin Administration in the Maintenance of Pregnancy in the Rat.

| Rat | Days Injected | Total R.U. Injected | Results |
|-------------------------------|---------------|---------------------|--|
| D38 | 16-18 | 198 | Aborted 19th day; fetus not recovered |
| D33 | 16-19 | 168 | " 20th " " dead |
| D41 | 16-19 | 168 | " 20th " " not recovered |
| D26 | 16-20 | 240 | " 21st " " living, not viable |
| D39 | 16-19 | 240 | " 21st " " " " |
| D23 | 16-20 | 240 | " 21st " " recovered, half eaten |
| D28 | 16-20 | 240 | " 21st " " living, viable |
| D30 | 16-21 | 336 | " 22nd " " " |
| D24 | 16-21 | 336 | " 22nd " " recovered, half eaten |
| D29 | 16-21 | 336 | " 22nd " " living, not viable |
| D43 | 16-22 | 456 | Sacrificed 24th day; dead, mummified fetus |
| D27 | 16-22 | 456 | " " " Uterus empty (large and hyperemic) |
| Control Animals—Not Injected. | | | |
| DX12 | | | Sacrificed 24th day; fetus living, viable |
| DX13 | | " " | " deformed, not viable |
| DX14 | | " " | " living, viable |
| DX15 | | " " | " " |
| DX16 | | " " | " " |

to deliver and upon autopsy on the 24th day the uterus of one was empty and that of the other contained a badly macerated fetus.

Series E. (Table II.) In this group of 15 animals, treatment was not instituted until the 18th day of pregnancy, *i. e.*, on the 3rd day after complete oophorectomy; dosages were given as in Series D and were continued when necessary until the 24th day. Four animals aborted on the 21st day, 6 on the 22nd, 2 on the 23rd. The remaining 3 failed to respond and upon sacrifice (26th day) 2 dead fetuses were recovered; the uterus of the third was empty. It is noteworthy that of the 21-day series 2 young were alive when aborted, 1 of which was viable; of the 22-ray animals, 3 were alive and viable.[†] In a sense, therefore, the abortion induced at or near the time of normal term simulated parturition, *i. e.*, in that live and viable young were delivered. The process of expulsion, when observed, seemed to differ in no way from that occurring during normal parturition.

TABLE II.
Influence of Delayed Oestrin Administration.

| Rat | Days Injected | Total R.U. Injected | Results on Fetus | |
|------------------------------|---------------|---------------------|----------------------|------------------------------------|
| E47 | 18-20 | 108 | Aborted 21st day; | living, viable |
| E51 | 18-20 | 108 | " 21st " | partly eaten |
| E56 | 18-20 | 108 | " 21st " | not recovered |
| E46 | 18-20 | 108 | " 21st " | living, viable |
| E50 | 18-21 | 168 | " 22nd " | " " |
| E48 | 18-21 | 168 | " 22nd " | " " |
| E59 | 18-21 | 168 | " 22nd " | not recovered |
| E52 | 18-21 | 168 | " 22nd " | living, viable |
| E58 | 18-22 | 240 | " 23rd " | not recovered |
| E61 | 18-21 | 168 | " 22nd " | dead, deformed |
| E54 | 18-23 | 336 | " 23rd " | living, not viable |
| E60 | 18-23 | 336 | " 23rd " | not recovered |
| E45 | 18-23 | 336 | Sacrificed 25th day; | cystic growth in uterus |
| E73 | 18-23 | 336 | " " | dead, macerated |
| E71 | 18-24 | 456 | " " | very large, dead |
| Control Animals—Not Injected | | | | |
| EX21 | | | Sacrificed 24th day; | living, viable |
| EX22 | | | " " | " " |
| EX25 | | | " " | (very large) |
| EX31 | | | " " | dead, macerated |
| EX33 | | | " " | living, badly deformed, non-viable |
| EX27 | | | " " | viable |

All animals were sacrificed immediately after uterine evacuation, the oophorectomies verified and the uteri examined. The latter were

* The practical difficulties involved in being on hand for every abortion resulted in failure to recover fetuses in many instances. It seems probable that some, at least, of the fetuses missed were alive at the time of expulsion but were promptly eaten—a characteristic cannibalism frequently displayed by the experimental female.

markedly hyperemic, large and well-nourished, comparing rather favorably with those of normal full-term females, and showing a marked contrast to the rigid, contracted tissues of untreated oophorectomized females. It is apparent that oestrin exercises some function during pregnancy, in the rat, in maintenance of the uterus and in this respect perhaps operates synergistically with progestin.

A positive response, when elicited, followed upon a continuous, and progressive increase in, oestrin dosage; abortion occurred during treatment and, with one exception, no response was evoked following withdrawal of oestrin administration. The marked variation in response, however, was striking. Thus, in Series A, 4 animals responded to a total of only 72 R.U., and 3 to 112; 3 failed to respond to a total of 160 R.U., however. In B, 5 responded to 140, 5 to 220, and in C, 3 responded to 108 and 2 to 168, whereas 10 animals failed to react to a total of 240 R.U. Variations in Series D and E are shown in the tables, where it will be seen that 4 animals failed to respond to 336 and to 456 R.U. The quantity required to produce an effect, apparently, is subject to marked individual variation, and the manner and the time of oestrin administration would appear to be of greater importance than the amount administered.

The foregoing observations suggest that oestrin in adequate, timely amounts appears to be definitely involved in preparation of the uterine structure for parturition; whether this consists in a sensitization to the action of some other agency or whether oestrin in itself initiates the birth mechanism remains to be determined.

8908 C

Lead Content of the Spinal Fluid with Special Reference to Multiple Sclerosis.

PAUL H. GARVEY AND FRED V. ROCKWELL. (Introduced by W. S. McCann.)

From the Department of Medicine, University of Rochester School of Medicine and Dentistry, and the Medical Clinics of the Strong Memorial and Rochester Municipal Hospitals.

Lead was suggested as an etiological factor in multiple sclerosis by Putnam.¹ Cone, *et al.*,² examined a series of 40 spinal fluids for

¹ Putnam, J. J., *Boston M. and S. J.*, 1883, **109**, 315; *J. Nerv. and Men. Dis.*, 1883, **10**, 446.

² Cone, W., Russel, C., Harwood, R. N., *Arch. Neurol. Psych.*, 1934, **31**, 236.

lead, using the qualitative hexanitrite test of Fairhall.³ In their series were 8 patients with multiple sclerosis. In 6 of these the tests for lead were positive. Rabinowitch, *et al.*,⁴ about the same time reported the results of tests of 50 spinal fluids, including those from 27 cases of multiple sclerosis. These authors used the Fairhall hexanitrite method and checked their results by spectographic determinations. These authors found positive tests for lead in only 2 of their 27 cases of multiple sclerosis; in 2 additional cases they obtained positive tests after administering ammonium chloride to their patients. Boshes⁵ tested 28 specimens of spinal fluid for lead, by the same method. Sixteen of his patients had multiple sclerosis; in only one of these did the spinal fluid yield a positive test for lead. All the above authors found occasional positive tests for lead among their control cases.

Because the above findings depended on a qualitative test that is difficult to control, we believed it would be worth while to investigate the lead content of a series of spinal fluids, using a precise quantitative micro-chemical method. Such a method has been devised by Wilkins, *et al.*⁶ This method involves the titrimetric extraction of lead with a standard solution of diphenylthiocarbazone in the presence of ammoniacal cyanide. The original method was designed for use with blood; we have adapted it to spinal fluids in the manner described below. All the precautions advocated by the last mentioned authors were closely observed.

Lumbar puncture was done with a dry, sterilized needle, and the fluid allowed to drop directly into a lead-free Pyrex flask. A measured volume of fluid, varying from 25-50 cc. was transferred to a Pyrex evaporating dish, and evaporated to dryness on a steam bath. The Pyrex dish was then transferred to an electric muffle furnace and ashed for 16 hours at 450°C. The ashed sample was dissolved in warm 5% nitric acid and transferred to a Pyrex separatory funnel, in which the titrimetric extraction was carried out. Simultaneous blanks were run with each set of determinations.

According to the above mentioned authors, the titrimetric-extraction method has a constant error of ± 0.001 mg. of lead. We have been able to reduce the lead in our blank determinations to less than 0.001 mg. We have further tested the method by adding known

³ Fairhall, L. T., *J. Biol. Chem.*, 1923, **57**, 455.

⁴ Rabinowitch, I. M., Dingwall, A., and MacKay, F. H., *J. Biol. Chem.*, 1933, **103**, 707.

⁵ Boshes, B., *Arch. Neurol. and Psych.*, 1935, **34**, 994.

⁶ Wilkins, E. S., Willoughby, C. E., Kraemer, E. O., and Smith, F. L., *Analytical Indust. and Eng. Chem.*, 1935, **7**, 33.

amounts of lead to fresh normal spinal fluids. The amounts added varied from 0.010 mg. to 0.050 mg. The maximum discrepancy between the amount added and amount recovered was noted in a fluid to which was added 0.050 mg. from which 0.047 mg. were recovered. In other recovery procedures carried out, the error was not more than ± 0.002 mg. of lead.

Nearly all our determinations were carried out upon samples, the volume of which exceeded 35 cc. We have classified as containing no lead those samples whose determined lead content, after subtraction of the blank, was less than our maximum error of ± 0.003 mg. This assumption we believe to be more than fair from a statistical viewpoint. Nearly all samples actually differed from the blank by less than 0.001 mg.

Using the method described, which we consider highly accurate and reliable, we have analyzed a series of spinal fluids from 42 patients. Our results are shown in Table I.

TABLE I.

| Type of Case | No. of Cases | Lead in Sample (mg.) |
|------------------------|--------------|-------------------------|
| Multiple Sclerosis | 9 | 0 |
| Lead Poisoning—chronic | 1 | 0 |
| ,, recovered | 2 | 0 |
| Control Cases | 28 | 0 |
| Case X* | 1 | 0.010 |
| Case Y* | 1 | 0.005 |
| Total | 42 | |

* Fluids collected without special precautions against lead contamination.

The absence of lead in the spinal fluids obtained from patients with multiple sclerosis does not necessarily eliminate lead as a causative factor of the disease. It is a well established fact that the barrier permeability for other heavy metals is low. That the same is true for lead is suggested by the negative findings in the case of chronic lead poisoning. A delicate test, such as that which we have used, will give a positive reaction for lead unless precautions are taken to avoid contamination.

Quantitative Estimation of Lactoflavin and of Vitamin B₆ in
Cow's Milk and in Human Milk.

PAUL GYÖRGY. (Introduced by Henry J. Gerstenberger.)

*From the Babies and Childrens Hospital and the Department of Pediatrics,
School of Medicine, Western Reserve University, Cleveland.*

It has been shown¹ that vitamin G as needed by the rat contains at least 2 major components: lactoflavin and vitamin B₆, the lack of the latter being associated with specific skin lesions ("rat pel-lagra" or, rather, "rat acrodynia"²).

By feeding to rats a vitamin B-free diet complemented by vitamin B proper, the antineuritic factor (B₁), and lactoflavin, or by vitamin B₁ and a vitamin B₆ concentrate, it was possible to obtain quantitative data^{3, 4} concerning the distribution of lactoflavin and vitamin B₆ in different foodstuffs. For the estimation of the lactoflavin values, the basal vitamin B-free diet was supplemented by crystalline (or highly purified) vitamin B₁ (3 international units daily) plus vitamin B₆ concentrate. For the estimation of vitamin B₆ values, vitamin B₁ was similarly provided but supplemented by pure lactoflavin (10 γ daily). The vitamin B₆ concentrate consisted of a yeast preparation such as may be obtained according to the method of Peters, *et al.*,⁵ by adsorption of a yeast extract on charcoal and subsequent elution with alcohol containing hydrochloric acid. It has been found that 1 cc. of this so-called Peters' eluate is equivalent to about 1 "rat-day dose" of vitamin B₆.

The values for lactoflavin were determined by means of the growth test, skin symptoms being too irregular and frequently insufficiently obvious. The "rat-day dose," taken as provisional unit, was defined as the minimum quantity of the substance which would give rise to a gain in weight of about 10 (9 to 11) gm. per week for at least 4 weeks.

The values for vitamin B₆ were based directly on the curative effect against dermatitis. The "rat-day dose," or provisional unit for estimation of vitamin B₆, therefore, was defined as the minimum quantity of the substance that would cause healing of the specific dermatitis.

¹ György, P., Kuhn, R., and Wagner-Jauregg, T., *Klin. Wehnschr.*, 1933, **12**, 1241; György, P., *Nature*, 1934, **133**, 498; György, P., *Biochem. J.*, 1935, **29**, 741.

² Birch, T. W., György, P., and Harris, L. J., *Biochem. J.*, 1935, **29**, 2330.

³ György, P., *Biochem. J.*, 1935, **29**, 760.

⁴ Kinnersley, H. W., O'Brien, J. R., Peters, R. A., and Reader, V., *Biochem. J.*, 1933, **27**, 225.

Average values for distribution of lactoflavin and vitamin B₆ in cow's milk tested on several occasions in the spring and summer of 1935 in Cambridge, England,³ are shown in Table I.

TABLE I.
Average Values for Distribution of Lactoflavin and Vitamin B₆ in Cow's Milk.*

| Daily Dose of Cow's Milk | Tests for Lactoflavin (Vitamins B ₁ and B ₆ provided): | | Tests for Vitamin B ₆ (Vitamin B ₁ and Lactoflavin provided): | |
|-----------------------------|--|-----|--|--------------------------------------|
| | Aver. Weekly Increase in Wt. of Rats | gm. | Aver. Weekly Increase in Wt. of Rats | gm. |
| cc. | | | | |
| 15 | | — | | 14 |
| 10 | | 12 | | 13 |
| 5 | | 4 | | 5 |
| | | | | Healing of Specific Dermatitis |
| | | | | Yes |
| | | | | , |
| | | | | Mostly none |

*Tests made in Cambridge, England.

These tests were repeated, in the winter and spring months of 1935-36 in Cleveland. The technic described was employed, as well as the same vitamin B₁, B₆ and lactoflavin supplements* used in the previous experiments in England.

The basal diet given the rats was as follows:

| | % |
|---------------------------------|----|
| Extracted casein | 18 |
| Cornstarch | 68 |
| Butterfat (melted and filtered) | 9 |
| Salt mixture (McCollum 185) | 4 |
| Cod liver oil | 1 |

TABLE II.
Average Values for Distribution of Lactoflavin and Vitamin B₆ in Certified and
Pasteurized Cow's Milk.*

| Material Fed | Tests for Lactoflavin (Vitamins B ₁ and B ₆ provided): | | Tests for Vitamin B ₆ (Vitamin B ₁ and Lactoflavin provided): | |
|---------------------------------------|--|--|--|--------------------------------------|
| | Amt. Given Daily | Aver. Weekly Increase in Wt. of Rats | Aver. Weekly Increase in Wt. of Rats | Healing of Specific Dermatitis |
| Raw certified cow's milk | cc. | gm. | gm. | |
| Raw certified cow's milk | 10 | 15.5 | 13 | Yes |
| | 5 | 10 | 7.5 | , |
| | 3 | 6 | 1 | No |
| Ordinary pasteurized cow's milk | 10 | 13 | 12 | Yes |
| | 5 | 8.5 | 7 | , |
| | 3 | 5 | 3 | No |

*Tests made in Cleveland.

* Kindly furnished by I. G. Farbenindustrie, Germany, through the courtesy of Winthrop Chemical Company, Inc., New York.

In the tests for vitamin B₆, cornstarch and extracted casein were replaced by sugar and Merck's unextracted casein, respectively. Graded doses of fresh milk (raw certified and ordinary pasteurized) were tested on from 4 to 6 rats at each level. The results are summarized in Table II.

When comparison is made between the values for lactoflavin and vitamin B₆ found in the milks tested in Cambridge and in Cleveland, it is seen that the "unit" (rat-day dose) for lactoflavin is distinctly lower for the Cleveland milk (5 cc.) than for the Cambridge milk (10 cc.); while for vitamin B₆ the level is at 5 to 10 cc. in each case, with the Cleveland milk somewhat favored.

The lactoflavin values for certified milk in Cleveland were slightly higher than those for pasteurized milk in Cleveland, but no conspicuous quantitative differences could be detected in the vitamin B₆ content of these milks.

Earlier workers⁵ have established the fact that vitamin B₁ in cow's milk and vitamin G in human milk are the limiting factors for rats.

TABLE III.
Average Values for Distribution of Lactoflavin and Vitamin B₆ in Human Milk.

| Material Fed | Amt. Given Daily | Tests for Lactoflavin (Vitamins B ₁ and B ₆ provided): | | Tests for Vitamin B ₆ (Vitamin B ₁ and Lactoflavin provided): |
|-----------------|------------------------|--|------|--|
| | | Aver. Weekly Increase in Wt. of Rats | gm. | |
| Human milk I | 15 | 15 | — | Yes ,, — |
| | 10 | 5 | 12 | |
| | 5 | 4 | 4 | |
| | 3 | 2 | — | |
| " " II | 15 | 7 | — | — — Yes No |
| | 10 | 4 | — | |
| | 5 | 2 | 6 | |
| | 3 | — | —1 | |
| " " III | 15 | 6 | — | — Yes ,, ,, |
| | 10 | 5 | 8 | |
| | 5 | 5 | 6 | |
| | 3 | — | 4 | |
| " " IV | 15 | 7 | — | — Yes ,, |
| | 10 | 5 | 11.5 | |
| | 5 | 5 | 6 | |
| Average of I-IV | | 8 | — | — Yes ,, Inconstant |
| | | 4.5 | 11 | |
| | | 5 | 5 | |
| | | 2 | 1.5 | |

⁵ Macy, I. G., Outhouse, J., Graham, A., and Long, M. L., *J. Biol. Chem.*, 1927, **73**, 189; Outhouse, J., Macy, I. G., Brekke, V., and Graham, A., *Ibid.*, p. 203; Sherman, H. C., and Axtmayer, J. H., *Ibid.*, 1927, **75**, 207; Hunt, C. H., and Krauss, W. E., *Ibid.*, 1928, **79**, 733; Macy, I. G., and Outhouse, J., *Am. J. Dis. Child.*, 1929, **37**, 379; Donelson, E., and Macy, I. G., *Am. J. Physiol.*, 1932, **100**, 420; Samuels, L. T., and Koch, F. C., *J. Nutrition*, 1932, **5**, 307.

In view of our recently acquired knowledge as to the complex nature of vitamin G, the question arises: Which component of vitamin G determines its low potency in human milk?

The milk of 5 lactating women was analyzed for content of lactoflavin and vitamin B₆. Three of the women gave sufficient amounts of milk for separate tests. The milk of the 2 remaining subjects was pooled and the mixture tested. As with cow's milk, graded doses of the fresh milk were tested, on from 3 to 4 rats at each level. The results are recorded in Table III.

While the vitamin B₆ content of human milk does not differ substantially from that of cow's milk (1 "rat-day dose" in 5 cc., and even less), the lactoflavin potency of cow's milk is on the average about 3 times as high as that of human milk. We have to conclude, therefore, that in human milk the lactoflavin represents the limiting factor. With the exception of one test, in all experiments with human milk the "unit" of lactoflavin could not be reached even when 15 cc. of the milk were given daily.

Under the conditions of the experiments here reported, the low vitamin G potency of human milk, expressed in terms of the whole vitamin G complex, can easily be rectified by adding pure crystalline lactoflavin in sufficient amounts.

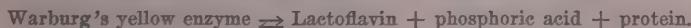
8910 C

Growth-Promoting Activity of Lactoflavin Administered Orally and Parenterally.

PAUL GYÖRGY. (Introduced by Henry J. Gerstenberger.)

*From the Babies and Childrens Hospital and the Department of Pediatrics,
School of Medicine, Western Reserve University, Cleveland.*

Lactoflavin is not only one constituent of the vitamin G (B₂) complex and as such a vitamin, but it is also a pro-ferment, being the prosthetic group of Warburg's yellow enzyme.¹ In the enzyme the lactoflavin is present in the form of a phosphoric acid ester:²



Lactoflavin exists in natural foodstuffs either in the free state or in the ester form, or even in the form of the colloidal protein

¹ Warburg, O., and Christian, W., *Biochem. Z.*, 1932, **254**, 438; *Naturwissenschaften*, 1932, **20**, 980; *Biochem. Z.*, 1933, **266**, 377.

² Theorell, H., *Biochem. Z.*, 1934, **275**, 37, 344.

compound. Rudy³ has shown that free lactoflavin can be phosphorylated by the action of intestinal phosphatase. Biologically inactive or less active flavins of the same group lack this property.⁴

Recently Laszt and Verzar⁵ demonstrated the interesting fact that when mono-iodoacetic acid was added to a biologically complete synthetic diet, the diet lost its growth-promoting quality, which could, however, be restored by the phosphoric acid ester of lactoflavin, though not by free lactoflavin. They concluded that mono-iodoacetic acid prevents the phosphorylation of the lactoflavin, probably within the intestinal wall, and, in consequence, the cellular synthesis of Warburg's yellow enzyme.

In view of these facts, the question arose, where in the organism the phosphorylation occurs; in particular, whether it is confined to the intestinal tract and, further, whether the phosphoric acid ester of lactoflavin parenterally injected would have a greater effect than free lactoflavin orally administered.

In order to answer these questions, we have compared the physiologic growth-promoting action of free lactoflavin and of pure (synthetic) lactoflavin-5-phosphoric acid* by administering each form orally as well as parenterally. The method used for the feeding experiments was that described previously by us.⁶ Slight modifications in the basal diet consisted in the substitution of extracted casein and cornstarch for caseinogen AB Glaxo and rice starch, respectively.

Graded doses were tested generally on 3 rats at each level. The

TABLE I
Average Values Obtained in Testing the Growth-Promoting Activity of Lactoflavin* by Oral and by Parenteral Administration.

| Material Fed | Amt. Given Daily | Aver. Weekly Increase in Wt. of Rats | |
|-----------------------------------|------------------------|---|---|
| | | When Lactoflavin Was Orally Administered | When Lactoflavin Was Parenterally Administered |
| Lactoflavin | 15 | gm. | gm. |
| | 10 | | 9 |
| | 9 | | 9 |
| | 7.5 | | 8.5 |
| Lactoflavin-5- phosphoric acid | 13.8 | 10.5 | 10 |
| | 6.4 | 7.5 | 9 |
| | 3.2 | 5 | 4.5 |

*Vitamins B₁ and B₆ provided.

³ Rudy, H., *Naturwissenschaft*, 1935, **23**, 286.

⁴ Kuhn, R., and Rudy, H., *Naturwissenschaft*, 1935, **23**, 286.

⁵ Laszt, L., and Verzar, F., *Poggens Arch. f. d. ges. Physiol.*, 1935, **226**, 693.

⁶ Kindly furnished by Professor R. Kuhn, Heidelberg, Germany.

⁶ György, P., *Biochem. J.*, 1935, **29**, 741.

results obtained are recorded in Table I. The values given represent averages.

Conclusions. The growth-promoting effect of lactoflavin or of lactoflavin-5-phosphoric acid is independent of the way in which it is administered, that is, whether orally or parenterally. There are no biological differences, as shown by growth tests, between lactoflavin and lactoflavin-5-phosphoric acid, the rat-day dose for both being about 7 to 10 γ. It can therefore safely be assumed that the phosphorylation of lactoflavin is not only an intestinal but also a general cellular reaction.

8911 C

Vitamin D Deficiency on Concentration of Blood and Tissue Enzymes of the Albino Rat.* V.

BARNETT SURE, M. C. KIK AND K. S. BUCHANAN. (With the technical assistance of James DeWitt.)

From the Department of Agricultural Chemistry, University of Arkansas.

Experimental rickets was produced according to the technique of Steenbock and Black,¹ with the modification of our paired feeding technique, so as to eliminate the influence of the plane of nutrition.² The animals were 29 to 55 days of age, and weighed 50 to 58 gm. at the beginning of the experiments. Since the ricketic type of diet employed allows only very small increases of weight, the size of the animals at the time of sacrificing yielded insufficient amount of blood for all the blood serum enzyme determinations; hence, some groups were taken for blood serum amylase and esterase, and others for blood serum phosphatase. Of the total numbers of groups studied, 24 showed by the line tests severe experimental rickets; 4, advanced; 8, moderate; and 2, mild.

There was a total of 658 titrations carried out in this investigation in duplicate, the results of which are summarized in Table I.

It will be noted that no noteworthy changes are apparent in concentration of blood and tissue enzymes in rickets developed in the albino rat compared with enzyme concentrations on the same diet supplemented with vitamin D supplied by irradiation of the ricketic ration.

* Research paper No. 428, Journal series, University of Arkansas.

¹ Steenboek, H., and Black, A., *J. Biol. Chem.*, 1924, **61**, 405.

² Sure, B., Kik, M. C., and Buchanan, K. S., *J. Biol. Chem.*, 1935, **108**, 27.

TABLE I.
Influence of Vitamin D Deficiency on Concentration of Blood and Tissue Enzymes.
P = Pathological; C = Control.

| Enzyme | No. of Groups Studied | Aver. for all groups | |
|----------------------|-----------------------|----------------------|-------|
| | | P | C |
| Blood Serum Amylase* | 17 | 31.9 | 32.9 |
| " " Esterase† | 18 | 17.2 | 18.4 |
| " " Phosphatase* | 27 | 62.0 | 58.2 |
| Trypsin* | 38 | 50.1 | 49.0 |
| Erepsin* | 38 | 24.3 | 24.2 |
| Pancreatic Amylase* | 38 | 280.0 | 265.0 |
| " Lipase‡ | 38 | 158.0 | 160.0 |
| Hepatic " | 38 | 26.9 | 27.9 |
| Pancreatic Esterase† | 38 | 13.1 | 13.6 |
| Hepatic " | 38 | 43.0 | 44.0 |

*expressed in units.

†expressed as mg. butyric acid.

‡expressed as mg. oleic acid.

The significant point in this study is the failure to obtain in experimental rickets large increases in concentration of blood serum phosphatase observed in human rickets.^{3, 4} As a matter of fact, Bodansky and Jaffe⁴ claim that the concentration of this blood enzyme is of even greater diagnostic value than blood phosphorus and calcium. It would appear then that experimental rickets is not the analogue of human rickets as it is generally assumed.

Our results on blood serum phosphatase in experimental rickets are in accordance with the recent findings of Scoz,⁵ the report of which appeared during the progress of our own investigations.

8912 C

Deficiency of Vitamins A and B Complex in Concentration of Blood and Tissue Enzymes of Albino Rat.* VI.

BARNETT SURE AND K. S. BUCHANAN. (With the technical assistance of James DeWitt.)

From the Department of Agricultural Chemistry, University of Arkansas.

We have reported¹ that in vitamin A deficiency there is a marked decrease in the concentration of blood serum esterase, an appreciable

³ Kay, H. D., *J. Biol. Chem.*, 1930, **89**, 249.

⁴ Bodansky, A., and Jaffe, H. L., *Am. J. Dis. Child.*, 1934, **48**, 1268.

⁵ Scoz, G., *Boll. Soc. Ital. Sper.*, 1935, **10**, 823.

* Research paper No. 429, Journal series, University of Arkansas.

¹ Sure, B., Kik, M. C., and Buchanan, K. S., *Am. J. Dig. Dis. and Nutr.*, 1936,

decrease in hepatic esterase, and a marked increase in hepatic lipase. In vitamin B₁ deficiency there is a marked decrease in the digestive efficiency of pancreatic esterase and a moderate decrease in the concentration of pancreatic and hepatic lipase, suggesting a disturbance in the digestion of fats in this avitaminosis. An appreciable increase in the concentration of blood serum phosphatase was found in a deficiency of the vitamin B complex.

In this communication we are submitting summarized data on the influence of multiple avitaminosis (deficiency of vitamins A and the B complex) on the concentration of blood and tissue enzymes of the albino rat. The animals were 6 to 9 weeks old and weighed 106 to 118 gm. at the beginning of the experiments. A total of 304 titrations were carried out in duplicate. The results are given in Table I.

TABLE I.
Influence of Deficiency of Vitamins A and the B Complex on the Concentration
of Blood and Tissue Enzymes.

| Enzymes | No. of groups studied | Aver. for all groups | | % decrease in patho-logical | % increase in patho-logical | % animal groups showing decrease | % animal groups showing increase |
|----------------------|-----------------------|----------------------|-------|-----------------------------|-----------------------------|----------------------------------|----------------------------------|
| | | P | C | | | | |
| Blood Serum Amylase* | 16 | 22.5 | 23.7 | 5.0 | — | 62 | 38 |
| " " Esterase† | 6 | 8.0 | 13.7 | 41.6 | — | 100 | 0 |
| Trypsin* | 19 | 54.0 | 62.0 | 12.9 | — | 53 | 47 |
| Erepsin* | 19 | 23.0 | 24.0 | 4.1 | — | 63 | 37 |
| Pancreatic Amylase* | 19 | 223.0 | 218.0 | 0.0 | 2.1 | 32 | 68 |
| " Lipase‡ | 19 | 118.0 | 166.0 | 28.3 | — | 90 | 10 |
| Hepatic | 18 | 22.7 | 22.4 | — | 1.0 | 47 | 53 |
| Pancreatic Esterase† | 18 | 8.6 | 16.0 | 46.2 | — | 94 | 6 |
| Hepatic | 18 | 21.1 | 24.6 | 17.0 | — | 66 | 34 |

*expressed in units.

†expressed as mg. butyric acid.

‡expressed as mg. oleic acid.

We conclude that the multiple avitaminosis of vitamins A and the B complex did not show increased disturbance in enzyme concentrations. The pronounced disturbance in blood serum esterase is due to vitamin A deficiency. The marked decrease in pancreatic esterase and the moderate decrease in hepatic lipase are to be credited to vitamin B₁ deficiency.

